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CONTENTS.

Vol. 13, No. 1.

SECTION I.

Protozoology.

SECTION II.

Virus Diseases.

SECTION III.

Bacteriology.

SECTION IV.

Parasitology.

SECTION V.

Mineral Metabolism and Deficiency.

SECTION VI.

Nutrition.

SECTION VII.

Toxicology and Poisonous Plants.

SECTION VIII.

Animal Industry: Wool Research.

Vol. 13, No. 2.

SECTION I.

Protozoology.

SECTION II.

Virus Diseases.

SECTION III.

Bacteriology.

SECTION IV.

Mineral Metabolism and Deficiency.

SECTION V.

Toxicology.

SECTION VI.

Nutrition.

SECTION VII.

Pathology.

SECTION VIII.

Wool Research.

SECTION IX.

Anatomy-Teratology.

VOLUME 13, No. 1.

Section I.

Protozoology.

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Ovine Anaplasmosis: The Transmission of *Anaplasma ovis* and *Eperythrozoon ovis* to the Blesbuck (*Damaliscus albifrons*).

By W. O. NEITZ, Section of Protozoology and Virus Diseases, Onderstepoort.

Introduction.

The transmission of *Anaplasma marginale* and *Anaplasma centrale* to several species of South African antelopes was discussed by Neitz and du Toit (1932) and Neitz (1935). The experiments showed that *A. marginale* produced an active infection in the blesbuck, the duiker and the blackwildebeest, whereas *A. centrale* resulted in a latent infection in the blesbuck.

According to Lignières (1919) *A. marginale* produces a latent infection in sheep and goats. This observation in sheep was confirmed by Kraus, Dois and Oyarzabal (1922), Sergent, Donatien and Lestoquard (1924) and Neitz and du Toit (1932). Theiler (1910) and de Kock and Quinlan (1926), on the other hand, failed to infect sheep with *A. marginale*. Their experiments were carefully carried out, and it must therefore be concluded that sheep cannot always become infected with bovine anaplasmosis.

De Kock and Quinlan (1926) and Donatien and Lestoquard (1930) showed that cattle are not susceptible to *A. ovis*.

On account of the difference in the behaviour of the three species of anaplasma in the ruminants mentioned above, experiments were undertaken to ascertain the response of the blesbuck to *A. ovis*.

Experimental Observations.

Three blesbuck (*Damaliscus albifrons*) were obtained through the kindness of the Provincial Administration of the Orange Free State from the Summerville Game Reserve at Theunissen. They arrived at Onderstepoort in May, 1936, and were kept in a comparatively tick free camp until they were needed. During the period that they were used for these experiments, they were kept in a stable. At first the animals were wild but they soon became so tame that the daily temperature and blood smears could be taken without great difficulty.

The Transmission of *Anaplasma ovis* and *Eperythrozoon ovis* to the Blesbuck.

The details about the subinoculations will be found in Table 1.

Experiment 1. (*S.* 6382.)

Object.—To ascertain whether one of the blesbuck harbours any blood parasites transmissible to sheep.

Method.—Blood from blesbuck 47080 was injected subcutaneously into two susceptible splenectomized sheep 41481 and 41596. Blood smears were examined daily for a period of 4 weeks.

Result.—No blood parasites could be demonstrated in either of the two sheep.

Conclusion.—Blesbuck 47080 did not harbour any blood parasites transmissible to sheep. It was assumed that since the blesbuck 47080 was free of parasites, that blesbuck 47086 and 47350 would also be free. Sufficient susceptible splenectomized sheep were not available to test each animal separately.

Experiment 2.

Object.—To transmit *A. ovis* and *Ep. ovis* to blesbuck 47080 and 47350.

Method.—Mixed blood of two sheep 40306 and 40326 harbouring *A. ovis* and *Ep. ovis* was injected into the two blesbuck.

Result.—Both antelopes reacted to *A. ovis* which was demonstrated on the 27th day in case of blesbuck 47080 and on the 32nd day in case of blesbuck 47350. Pronounced anaemia was observed clinically and microscopically in both animals. In neither of the animals could *Ep. ovis* be demonstrated microscopically. The temperatures remained normal (99-102° F.) throughout the period of observation. There was a gradual loss of condition and general weakness even after the disappearance of the parasites from the blood stream.

Blesbuck 47080 died 107 days after infection. At post-mortem this animal showed emaciation, anaemia, acute gastro-enteritis, oedema of the lungs, gelatinous infiltration of the fat and a *Bunostomum trigonocephalum* infestation.

Blesbuck 47350 died 103 days after receiving the infected blood. At autopsy ascites, hydrothorax, hydropericard, subendocardial haemorrhages, cachexia, gelatinous infiltration of the fat and oxyuriasis were observed.

Conclusion.—*A. ovis* could be transmitted to both antelopes. *Ep. ovis* could not be demonstrated microscopically, but may have been present as a latent infection. It is not clear to what extent the verminosis influenced the degree of anaemia. Both animals died several weeks after the disappearance of the parasites from the blood stream. It is possible that under these unnatural conditions the anaplasmosis infection was indirectly responsible for the deaths.

Experiment 3.

Object.—To confirm the microscopic diagnosis of *A. ovis* made in the previous experiment, by injecting blood from the antelopes into sheep, calves and a blesbuck.

Method.—(*a*) Blood from blesbuck 47080 was injected into two splenectomized sheep 41481 and 41596 used in experiment No. 1, into calf 7316, and into blesbuck 47086.

(*b*) Blood from blesbuck 47350 was injected into calf 7364.

Result.—(*a*) Sheep 41481 reacted to *A. ovis*, but three days after the appearance of the parasites, the animal died from meteorism of the rumen. *Ep. ovis* could not be demonstrated in this animal, but would probably have appeared had the animal lived longer, since this animal was known to be fully susceptible. Sheep 41596 reacted to *A. ovis* and *Ep. ovis*. A very marked anaemia developed as the result of the mixed infection, and the animal died eight days after the first appearance of the parasites.

Blesbuck 47086 reacted to *A. ovis*, but *Ep. ovis* could not be demonstrated microscopically. The infection did not produce a febrile reaction. The animal died 14 days after the first appearance of the parasites. At autopsy there were anaemia, icterus and a light *Haemonchus contortus* infection present.

Calf 7316 did not react to *A. ovis*. Three months later this calf was injected with *A. marginale* to which it reacted 21 days later.

Calf 7364 did not react to *A. ovis*. Six weeks later blood from this calf was injected into three sheep which failed to react to *A. ovis*. These sheep were later found to be susceptible to *A. ovis*. The calf reacted to *A. marginale* on testing its susceptibility.

Conclusion.—It was possible to confirm the microscopic diagnosis of *A. ovis* and to demonstrate a latent infection of *Ep. ovis* in one of the blesbuck by subinoculation of blood into a susceptible splenectomized sheep. The second splenectomized sheep died, and would probably also have reacted to *Ep. ovis* since it was fully susceptible.

Both the inoculated calves failed to react to *A. ovis*, and they were subsequently found to be susceptible to *A. marginale*.

Discussion.

In the appended Table II, an attempt is made to show the different characteristics of the anaplasma of the ruminants. It will be noticed that the information on this subject is incomplete, but nevertheless a number of important points are available, especially those on the susceptibility of the different species of the family

TABLE 1. (S. 6382.)

D.O.B. Number of Animal.	Injected from.	Object.	Dose of Blood.
Blesbuck 47080......	Sheep 40306 and 40326 both carriers of *A. ovis* and *Ep. ovis.*	To transmit the parasites of the donors to the blesbuck	10 c.c. i.v., 10 c.c. subcut
41481. Susceptible splenectomized sheep	Blesbuck 47080 before receiving infective dose	To ascertain whether blesbuck harbours any blood parasites	10 c.c. i.v.....
	Blesbuck 47080 while showing *A. ovis* in the blood	To confirm the diagnosis of *A. ovis* in blesbuck	10 c.c. i.v.....
41596. Susceptible splenectomized sheep	Blesbuck 47080 before receiving infective dose	To ascertain whether blesbuck harbours any blood parasites	10 c.c. i.v.....
	Blesbuck 47080 while showing *A. ovis* in the blood	To confirm the diagnosis of *A. ovis* in the blesbuck	10 c.c. i.v.....
Blesbuck 47086.....	Blesbuck 47080 while reacting to *A. ovis.*	To transmit *A. ovis* to blesbuck..........	10 c.c. i.v.....
Bovine 7316........	Blesbuck 47080 while reacting to *A. ovis*..	To exclude the possibility of *A. marginale* infection in the blesbuck	10 c.c. i.v.....
	Bovine 7319, a carrier of *A. marginale*....	To ascertain whether Bovine 7316 is susceptible to *A. marginale*	10 c.c. i.v.....
Blesbuck 47350.....	Sheep 40306 and 40326, both carriers of *A. ovis* and *Ep. ovis*	To transmit the parasites of the sheep to blesbuck	10 c.c. i.v.....
Bovine 7364........	Blesbuck 47350 while reacting to *A. ovis*..	To exclude the possibility of *A. marginale* infection	10 c.c. i.v.....
	Calf 7316, a carrier of *A. marginale*......	To ascertain whether bovine 7364 is susceptible to *A. marginale*	10 c.c. i..v....
Sheep 52872........	Bovine 7364..........................	To ascertain whether bovine 7364 harbours a latent infection of *A. ovis*	10 c.c. i.v.....
	Sheep 41835, 47079 and 46053..........	To ascertain whether sheep 52872 is susceptible to *A. ovis*	10 c.c. i.v.....
Sheep 52683........	Bovine 7364..........................	To ascertain whether bovine 7364 harbours a latent infection of *A. ovis*	10 c.c. i.v.....
	Sheep 41835, 47079 and 46053..........	To ascertain whether sheep 52683 is susceptible to *A. ovis*	10 c.c. i.v.....
Sheep 52737........	Bovine 7364..........................	To ascertain whether bovine 7364 harbours a latent infection of *A. ovis*	10 c.c. i.v.....
	Sheep 41835, 47079 and 46053..........	To ascertain whether sheep 52737 is susceptible to *A. ovis*	10 c.c. i.v.....

TABLE 1. (S. 6382)—*continued.*

Date.	Result. Incubation Period in Days.	*Anaplasma Ovis.*	Incubation Period in Days.	*Eperythrozoon Ovis.*
7/1/1938	27	Parasites appeared in large numbers. At the beginning of the experiment the erythrocyte count was 6 million, and the 14th day after the first appearance of the parasites, the erythrocyte count had dropped to 1·5 million. The blood showed basophilia, polychromasia anisocytosis and Jolly bodies. Parasites could be demonstrated for a period of 60 days. Blesbuck died 24/4/1938	—	*Ep. ovis* could not be demonstrated microcopically. The presence of a latent infection was shown by sub-inoculating blood into sheep 41596.
7/1/1938	—	No blood parasites could be demonstrated..........		—
4/2/1938	21	*A. ovis* could be demonstrated for 3 days. Sheep died from hoven 27/2/1938	—	*Ep. ovis* did not appear.
7/1/1938	-	No blood could be demonstrated..................	—	-
4/2/1938	15	*A. ovis* could be demonstrated for 8 days. Severe anaemia developed and sheep died 25/2/1938	15	*Ep. ovis* appeared in large numbers and undoubtedly complicated *A. ovis* infection.
17/2/1938	20	*A. ovis* appeared in large numbers. Animal died 21/3/1938	—	*Ep ovis* could not be demonstrated microscopically.
26/2/1938	—	No parasites seen in blood smears for a period of 100 days	—	—
18/5/1938	21	A typical *A. marginale* infection was observed......	—	-
26/4/1938	32	*A. ovis* appeared in large numbers, and produced marked anaemia. Parasites seen for a period of 40 days. Animal died 6/8/1938		*Ep. ovis* could not be demonstrated microscopically.
8/7/1938	-	No blood parasites appeared for a period of 8 weeks..	—	—
2/9/1938	12	A typical *A. marginale* infection was observed......	—	—
22/8/1938	—	Did not react to *A. ovis*.........................	—	Did not react to *Ep. ovis.*
26/9/1938	15	Reacted to *A. ovis*.............................	15	Reacted to *Ep. ovis.*
22/8/1938	—	Did not react to *A. ovis*.........................	—	Did not react to *Ep. ovis.*
26/9/1938	15	Reacted to *A. ovis*.............................	15	Reacted to *Ep. ovis.*
22/8/1938	—	Did not react to *A. ovis*.........................	—	Did not react to *Ep. ovis.*
26/9/1938	30	Reacted to *A. ovis*.............................	—	Did not react to *Ep. ovis.*

TABLE 2.

The Behaviour of the Three Species of Anaplasma in Ruminants.

Characteristics.	Parasite.	Cattle.	Sheep.	Goats.	Blesbuck (*Damaliscus albifrons*).	Blackwildebeest (*Conochaetes gnu*).	Duiker (*Sylvicapra grimmi*).
Incubation period in days	*A. marginale*.	15–25	Latent infection	Latent infection	25	38	35
	A. centrale...	21–42	?	?	Latent infection	?	?
	A. ovis......	Not susceptible	20–35	20–35	20–32	?	?
Nature of infection...	*A. marginale*.	Active infection	Latent infection	Latent infection	Active infection	Active infection	Active infection.
	A. centrale...	Active infection	?	?	Latent infection	?	?
	A. ovis......	Not susceptible	Active infection	Active infection	Active infection	?	?
Situation of the parasites in the erythrocytes	*A. marginale*.	90 Per cent. marginal forms	Latent infection	Latent infection	90 Per cent. marginal forms	90 Per cent. marginal forms	90 Per cent. marginal forms.
	A. centrale...	88 Per cent. central forms	?	?	Latent infection	?	?
	A. ovis......	Not susceptible	65 Per cent. marginal forms	65 Per cent. marginal forms	65 Per cent. marginal forms	?	?
Number of erythrocytes that may be parasitized	*A. marginale*.	5–25 per cent..	Latent infection	Latent infection	5 per cent.	2 per cent.	2 per cent.
	A. centrale...	5–25 per cent.	?	?	Latent infection	?	?
	A. ovis......	Not susceptible	5–25 per cent.	5–25 per cent.	7·5 per cent.	?	?
Period in days during which parasites could be demonstrated microscopically	*A. marginale*.	10–40	Latent infection	Latent infection	14	17	14
	A. centrale...	10–40	?	?	Latent infection	?	?
	A. ovis......	Not susceptible	20–60	20–60	40–60	?	?
Clinical symptons.....	*A. marginale*.	May be very severe	No symptons	No symptons..	No symptoms..	No symptons..	No symptons.
	A. centrale...	Mild reaction..	?	?	No symptoms..	?	?
	A. ovis......	Not susceptible	As a rule mild.	As a rule mild.	Mild symptoms	?	?

Cavicornidae. The susceptibility of the bovine and the blesbuck to the three species of Anaplasma has been fully worked out, whereas in the other ruminants this information is still lacking. Cattle are susceptible to *A. marginale* and *A. centrale* only. The blesbuck is susceptible to all the three species of anaplasma. In this antelope *A. marginale* and *A. ovis* produce active infections, whereas *A. centrale* results in a latent infection. With this information one should be able to isolate *A. marginale* in a pure state from a blesbuck that harbours a mixed infection of *A. marginale* and *A. ovis* by passaging the blood through cattle. On the other hand no method can be indicated for obtaining a pure strain of *A. ovis* from such a mixed infection, because all the ruminants mentioned in Table II are susceptible to *A. marginale.*

Another important fact that has been demonstrated is that the passage of these organisms through the various species of ruminants does not influence their chief characters, viz., their situation in the erythrocytes and their morphological structure.

CONCLUSIONS.

1. *Anaplasma ovis* produces an active infection in the blesbuck. The incubation period varies from 20 to 32 days. The parasites can be demonstrated microscopically for a period of 40 to 60 days. Anaemia resulted from the infection.

2. A latent infection of *Eperythrozoon ovis* in one of the blesbuck was demonstrated by subinoculination of blood into a susceptible splenectomized sheep.

3. The three blesbuck died. It is however not clear whether the anaplasmosis reaction was indirectly responsible for the deaths.

4. It was not possible to transmit *A. ovis* from the blesbuck to the cattle.

5. *A. ovis* did not change its characteristic morphology by passage through the blesbuck.

6. There is good reason to believe that blesbuck can act as reservoirs of *A. ovis* under natural conditions.

REFERENCES.

DE KOCK, G., AND QUINLAN, J. (1926). Splenectomy in domesticated animals and its sequelae, with special reference to Anaplasmosis in sheep. *11th and 12th Repts., Dir. Vet. Educ. and Research*, Pt. 1, pp. 369-480.

KRAUS, R., DIOS, R., AND OYARZABAL, J. (1922). Ueber ein unsichtbares Stadium bei pathogene Protozoen. (Piroplasma, Anaplasma und Trypanosomen). *Münch. Med. Woch.*, Vol. 68, No. 28, pp. 867-868.

LIGNIÈRES, J. (1919). Contribution à l'étude de l'anaplasmose bovine. Expériences d'inoculation de *Anaplasma argentinum* à plusieurs espèces animales. Les moutons et les chèvres sont nettement réceptifs. *Bull. Soc. Path. Exot.*, Vol. 12, No. 8, pp. 641-651.

NEITZ, W. O., AND DU TOIT, P. J. (1932). Bovine Anaplasmosis: A method of obtaining pure strains of *Anaplasma marginale* and *Anaplasma centrale* by transmission through antelopes. *18th Rept. Dir. Vet. Serv. and Anl. Indust., Union of South Africa*, pp. 3-20.

NEITZ, W. O. (1935). Bovine Anaplasmosis: The transmission of *Anaplasma marginale* to a black wildebeest. (*Conochaetes gnu.*) *Onderstepoort Jnl. of Vet. Sci. and An. Ind.*, Vol. 5, No. 1, pp. 9-11.

SERGENT, E., DONATIEN, A., PARROT, L., LESTOQUARD, F., PLANTUREUX, E., AND ROUGEBIEF, H. (1924). Inoculation au mouton de l'anaplasme du boeuf. *Bul. Soc. Path. Exot.*, Vol. 17, No. 4, pp. 295-298.

THEILER, A. (1910). Texas-fieber, Rotwasser und Gallenkrankheit der Rinder. *Zeitsch. für Infektionskrh. der Haustiere.*, Vol. 8, No. 1, pp. 39-62.

Section II.

Virus Diseases.

ALEXANDER, R. A., MASON, J. H. AND NEITZ, W. O.	Studies of the Rickettsias of the Typhus-Rocky-Mountain-Spotted-Fever group in South Africa I. Isolation of strains.
ALEXANDER, R. A., AND MASON, J. H.	Studies of the Rickettsias of the Typhus-Rocky-Mountain-Spotted-Fever group in South Africa II. Morphology and cultivation.
MASON, J. H. AND ALEXANDER, R. A.	Studies of the Rickettsias of the Typhus-Rocky-Mountain-Spotted-Fever group in South Africa III. The disease in the experimental animal. Cross-immunity tests.
MASON, J. H. AND ALEXANDER, R. A.	Studies of the Rickettsias of the Typhus-Rocky-Mountain-Spotted-Fever group in South Africa IV. Discussion and classification.

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Studies of the Rickettsias of the Typhus-Rocky-Mountain-Spotted-Fever Group in South Africa. I.—Isolation of Strains.

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RESEARCH work into heartwater, a disease of sheep, goats, and cattle caused by *Rickettsia ruminantium* (Cowdry 1926) has been hampered to a very great extent by the lack of a susceptible small laboratory animal. Up to the present all efforts to infect the guinea-pig, rabbit, rat, and mouse have been quite unsuccessful. It was, therefore, decided to procure one or more strains of the typhus group of rickettsias, which are pathogenic for guinea-pigs, to serve as a positive control for certain technique and experimental methods. During the course of this work other rickettsias were encountered and ultimately were compared with a strain of fièvre boutonneuse kindly supplied by Dr. Balozet of Tunis. The present series of papers is presented to record the observations that have been made, in the hope that the data may be of value in helping to clarify the somewhat confused position of the South African rickettsias in the current literature.

A. STRAIN "APPLETON".

On May 5th, 1936, an assistant reported that a dog, belonging to him, had developed biliary fever and made a request for the animal to be treated. The history supplied was that 9 days previously the dog, a smooth-haired Fox Terrier puppy about 8 months old, had accompanied the owners to a picnic held on the banks of a river some sixteen miles north of Pretoria. During the following week many partially engorged ticks were noticed on the animal; these were removed from time to time by hand and were destroyed without identification. The dog then became ill; the symptoms described were listlessness anorexia, anaemia, and fever. In spite of the history, which strongly supported a diagnosis of infection with *Piroplasma canis*, treatment was refused pending an accurate diagnosis and the owner was instructed to submit bloodsmears on the following day. A thorough examination of these preparations, stained by Giemsa, failed to reveal the presence of any piroplasms, nor was any sign of anaemia apparent. The owner was then instructed to present the dog for clinical examination. The puppy,

when seen the following morning, was in good condition but dull and disinclined to run about; temperature 103·5° F.; respiration slightly accelerated; pulse rapid, full, and not bounding; conjunctiva pink; urine normal; slight watery discharge from the eyes and nostrils; in addition it was reported that the patient had vomited several times during the night and had developed diarrhoea. Blood-smears were taken and revealed the presence of an occasional basophilic and polychromatic erythrocyte but no piroplasms. In the afternoon the temperature had risen to 105° F. so blood was taken from the jugular vein and injected, in 3 c.c. amounts, intraperitoneally into each of 3 guinea-pigs. The dog was returned with a tentative diagnosis of distemper and symptomatic treatment prescribed. Two days later it was reported that the dog had made an uneventful recovery.

The following is the history of the inoculated guinea-pigs:—

1. *Guinea-pig* 1 showed a sharp rise in temperature to 105° F. on the afternoon of the 4th day; on the following morning the temperature had returned to ±103° about which level it fluctuated for 7 days when the animal was killed by ether anaesthesia and a saline emulsion of the brain divided equally between two guinea-pigs by intraperitoneal injection.

2. *Guinea-pig* 2.—The temperature fluctuated slightly below 103° F. for 10 days and then rose to 104° F. As the guinea-pig appeared to be somewhat dull and flaccid it was killed and the brain injected intraperitoneally into two more guinea-pigs.

3. *Guinea-pig* 3.—During a period of 4 weeks, showed no rise in temperature nor apparent departure from normal health and was discharged.

Post-mortem examination of the two sacrificed guinea-pigs showed no abnormality other than a slight but definite enlargement of the spleen.

All four guinea-pigs of the second generation showed a marked febrile reaction reaching a maximum of 105·2° F. after an incubation period of four days. They were killed on the 10th day after injection when the temperature showed a tendency to drop and the condition, subsequently shown to be due to a rickettsia (see Part 2), was maintained by brain-peritoneum sub-inoculation.

B. Strain "Hare".

On August 1st, 1936, a grey hare (*Lepus saxatilis*) was shot on a farm in the neighbourhood of Premier Mine in the Pretoria District. The hare was placed in a large china basin overnight and on the following morning the ticks which had detached were collected. A total of 12 partially engorged nymphae, identified as *Hyalomma aegyptium* var, *impressum,* were placed on the back of a guinea-pig being confined by a wire gauze cage held in position by

adhesive calico. Only 5 ticks attached and engorged to repletion, the remaining 7 being found dead in the gauze cage; engorgement was completed in 8 days (5.8.36 to 13.8.36). The guinea-pig showed no febrile reaction but from the 7th to 9th day after the attachment of the ticks it was noticed to be dull. On the 9th day it was killed by ether anaesthesia and a saline emulsion of the entire brain was divided between two guinea-pigs, the inoculum being given intraperitoneally. Both guinea-pigs developed a febrile reaction after an incubation period of 4 days and the disease, subsequently shown to be due to a rickettsia (see Part 2), has been maintained by brain-peritoneum inoculation from that time.

C. Strain "Robertson". (Tick Bite Fever.)

This strain was obtained from Dr. J. H. S. Gear of the South African Institute for Medical Research, Johannesburg, who in collaboration with Dr. Bevan (1936) has published a preliminary report on the disease in man. It was isolated from the blood of a human patient who became ill after being associated with the deticking of dogs. According to Gear (personal communication) confirmed by a limited number of cross-immunity tests by us, the strain is immunologically identical with several other strains he isolated from human cases on the Witwatersrand. Moreover, Gear and Douthwaite (1939) reported complete reciprocal cross-immunity with an additional strain isolated from a dog tick *Haemaphysalis leachi*.

D. Strain Rat Typhus.

This strain also was obtained from Dr. Gear. Originally it was isolated by Dr. M. F. Finlayson, Public Health Laboratory, Capetown, who supplied the following particulars:—

> "The strain of rat typhus which I gave to Dr. Gear was isolated from the brains of wild rats caught on a farm in the Klein Drakenstein area near Paarl in November, 1935. A case of typhus had occurred on this farm and I saw the case in convalescence but was unable to isolate virus from the blood. Apparently a focus of rat typhus infection occurs in this area as three years earlier a case occurred near Paarl and a strain of murine typhus was isolated from rats by Dr. Rhodes. My strain and that of Dr. Rhodes appear to be identical by cross-immunity tests and reactions. The strain has been passaged in guinea-pigs at ten-day intervals until recently when it was discontinued voluntarily. Takes have been most regular and I have no reason to believe that the strain has altered in virulence since its isolation."

E. Fièvre boutonneuse.

The strain of fièvre boutonneuse was isolated from ticks, *Rhipicephalus sanguineus*, kindly supplied by Dr. L. Balozet of the Pasteur Institute, Tunis. The ticks, which had been collected from dogs, were forwarded by ordinary mail in what appeared to be a section of bamboo or cane plugged with cork.

To establish infection in guinea-pigs two methods were adopted:

(*a*) Intraperitoneal injection of saline emulsion of whole ticks.

(*b*) Feeding ticks.

(*a*) *Injection of tick emulsion.*—This was the method adopted with success by Pijper and Dau (1936). Before emulsification the ticks were washed in ether for several minutes (ether changed twice) then in sterile saline (changed 4 times); they were then ground up with sterile precautions in a mortar, the requisite amount of saline being added in small quantities from time to time. To eliminate coarse particles the emulsion was spun lightly at 1,500 revs. per minute for 1 minute and the supernatant fluid was immediately injected intraperitoneally into guinea-pigs. The guinea-pigs were temperatured twice daily and subinoculations were made at any time between the 7th and 18th day when a febrile reaction appeared to warrant it. For subinoculation an emulsion of brain only was used, the dose for each guinea-pig being not less than one-quarter of a brain.

Altogether 3 attempts were made to establish a strain in this way without success, as follows:—

1. On 11.10.37 200 completely or partially engorged nymphae, immediately on arrival from Tunis, were emulsified and divided among 6 guinea-pigs. Serial passage through guinea-pigs was carried out for 11 generations without establishing infection, and the attempt was abandoned.
2. On 13.11.37 it was found that 50 nymphae, which had been maintained in a constant humidity (80 per cent.) and temperature (80° F.) room, had recently moulted to adults. These ticks were fed on 4 guinea-pigs; after being attached for 5 days (i.e. partially engorged) they were removed, emulsified and the emulsion divided intraperitoneally among 4 guinea-pigs. Subinoculations were carried out in the usual manner for 4 generations but infection was not established.
3. On 2.12.38 an emulsion of approximately 50 adults and nymphae of a second batch of ticks received from Tunis was divided intraperitoneally among 3 guinea-pigs. On the 5th day after injection one of these guinea-pigs commenced a febrile reaction, which attained a maximum of 105·2° F. on the 8th day when the brain was subinoculated into 4 guinea-pigs. All the guinea-pigs of the second generation succumbed to peritonitis as a result of bacterial infection.

(*b*) *Feeding Ticks.*—On 11.11.37 about 12 adults were placed on the backs of each of 5 guinea-pigs. The ticks attached readily. All the guinea-pigs showed a slight febrile reaction between the 6th and 10th day after tick infestation though only in one case did the temperature rise above 105° F. Two, which showed the most severe reaction, were subinoculated on the 10th and 11th day respectively, with the following results:—

1. All three guinea-pigs succumbed to peritonitis of bacterial origin.

2. Both guinea-pigs commenced a febrile reaction on the 6th day after injection, the temperature rising to a point above 105° F. on the 9th and 10th day respectively. Both guinea-pigs were destroyed for serial passage on the 10th day and the disease has been maintained by serial brain-peritoneum inoculation.

Comment.—The demonstration of rickettsia in smears prepared from the tunica vaginalis of the testes has indicated that a rickettsial infection is being maintained. Morphologically these rickettsias closely resemble those described by Hass and Pinkerton (1936). In spite of this it is admitted readily that there is no direct experimental evidence to prove that the rickettsia infection isolated is that to which the name fièvre boutonneuse has been applied in the literature. However, there is little reason to doubt the contention for the following reasons:—

1. Balozet informed us that no strain of fièvre boutonneuse is maintained in guinea-pigs in the laboratory at Tunis. Whenever a strain is required for experimental purposes it is isolated from ticks (*R. sanguineus*) collected from dogs at the Institute. It is from these ticks that our strain was established.
2. We have carried out sufficient work at Onderstepoort, involving the serial passage of guinea-pig brain material in guinea-pigs to be reasonably certain that the available stock of guinea-pigs do not harbour a transmissible rickettsial infection.

For these reasons we have not hesitated to label the strain "fièvre boutonneuse". It is quite possible that minor differences in respect of some properties may be established when compared with other strains described in detail in the literature but for the purpose of these studies the North African strain was the one which could be most easily obtained.

Summary.

Details are given of the origin and methods of isolation of five strains of rickettsia.

1. Strain "Appleton" from a dog.
2. Strain "Hare" from ticks *Hyalomma aegyptium* collected from a hare (*Lepus saxatilis*).
3. Strain "Robertson" (Tick-bite fever) from a human patient.
4. Rat Typhus from a rat.
5. Fièvre boutonneuse from ticks (*Rhipicephalus sanguineus*) collected from dogs in Tunis.

Bibliography.

See conclusion of Part IV.

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Studies of the Rickettsias of the Typhus-Rocky-Mountain-Spotted-Fever Group in South Africa.

II.—Morphology and Cultivation.

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In the previous article (this journal) details were given of the source and method of isolation of five strains of rickettsia. These strains have been maintained either by serial passage in guinea-pigs, or by cultivation on the chorio-allantoic membrane of the chick embryo, or by both methods. In this paper the microscopic appearance of the scrotal exudate and method of egg-membrane cultivation will be described and the morphology of the rickettsias compared particularly in view of Pinkerton's classification of this group of organisms. (Pinkerton 1936.)

Technique.

Smears of the exudate were made by scraping the surface of the retracted testicle, or the tunica vaginalis of the scrotum firmly but gently with a sharp scalpel, transferring the resulting fluid material to the edge of a clean glass slide and then drawing the film in the usual way. Similar preparations were made from the surface of the spleen and the parietal peritoneum.

Smears were fixed with May-Grünwald and stained with Giemsa (2 drops of Hollborn's Giemsa per c.c. of M/500 phosphate buffer pH 7·4 for 30 minutes). Differentiation in dilute acetic acid was not done but the stained films were washed very thoroughly in running water before being dried for examination. For the demonstration of intranuclear rickettsias it was found advantageous to differentiate overstained preparations by exposure to diffuse sunlight for several days. In our hands Lepine's or Castaneda's stains were found to be quite unsatisfactory, but later a staining method recommended by Pinkerton (personal communication) has been used with success. Since no reference to the method has been noticed in the literature up to the present time the technique is detailed with due

acknowledgement of the source. Smears are fixed by very gentle heat fixation or preferably only by thorough air drying. Stain with 0·25 per cent. aqueous solution of basic fuchsin for 3 to 5 minutes. Pour off the stain and without washing decolorize with 0·5 per cent. citric acid for about 5 seconds. Wash in water and counterstain with 1 per cent. aqueous methylene blue for 10 seconds. It is preferable to filter the basic fuchsin on to the slide through filter paper and to apply the citric acid from a pipette. The rickettsias stain bright red, the cellular material blue. This method has been of little value for morphological studies but it has been exceedingly useful for the rapid examination of preparations since it is quite remarkable with what rapidity rickettsias may be detected and an estimate of the degree of rickettsial infection obtained.

Microscopic Appearance of the Exudate.

The general microscopic picture with all strains was practically identical. In the early stages there was a marked increase in the number of neutrophiles and eosinophiles followed by a relative increase in the number of large mononuclear cells other than serosa cells. Then serosa cells in various stages of mitosis made their appearance, and many were seen with a vacuolated cytoplasm. By the end of the second day after the commencement of the scrotal reaction cellular phagocytosis had commenced. This was always a prominent feature and by the time the scrotal swelling had begun to decrease it was common to find 4 or 5 cells per oil immersion field literally crammed with erythrocytes, neutrophiles or eosinophiles and even containing monocytes. As the reaction passed off the degree of promiscuous phagocytosis decreased, the number of polymorphonuclear cells, monocytes, mitotic and vacuolated serosa cells progressively diminished until the picture had returned to normal.

Rickettsias could be demonstrated regularly only in the early stages of the clinical scrotal reaction. Before this stage it was rare to find them, and later, when cellular phagocytosis was marked, a prolonged search was invariably fruitless. This observation is of interest in view of the high infectivity of testicular washings in the later stages of the reaction. Attention must be directed to one peculiarity which was observed constantly and that is the presence of a fairly fine, reddish-brown-staining, granular deposit which might be so voluminous as to obscure the general picture. The origin of this deposit and its nature is quite obscure, but careful control showed that it did not originate from either the fixative, the stain or the glass slides.

Morphology of the Rickettsias.

Strain "Hare".—Rickettsias were always exceedingly rare and could be demonstrated only in the initial stages of the scrotal reaction; even then it was frequently necessary to search through a well prepared and properly stained preparation for more than half-an-hour before finding 2 or 3 cells containing organisms. Typically the rickettsias are intracellular; extracellular forms have been found

but it seems probable that this location is due to rupture of cells during the process of drawing the film. The parasitized cells were of two different types:—

1. A large cell with a large light purple-staining, oval-shaped nucleus containing several well-defined nucleoli, i.e., a serosa cell.
2. A smaller cell with a more compact darker-staining and usually indented nucleus, i.e., a monocyte.

The exact nature of the cells was not determined either by supravital staining or by the injection of India ink.

The rickettsias were never clumped together nor aggregated in the form of large masses of organisms to distend the cytoplasm or displace the nucleus. On the contrary they were irregularly scattered throughout the cytoplasm in numbers which permitted easy counting, the usual number being 6 to 14 though 30 or more have been found. (*Cf.* Plate 1, fig. 1 and 1A.) In spite of a very careful search, intranuclear organisms have never been seen in preparations from the guinea-pig. The morphology of individual organisms is typical. In stained smears the colour tends to the bluish side of purple and there is a characteristic " soft " appearance in contrast to the " hard " metallic lustre of granules of the polymorphonuclear cells. Usually the shape is lanceolate but all forms varying from paired cocci to elongated bacilli have been seen. No tendency to chain formation was observed at any time. Attention must be directed to the observation that, surrounding the large majority of individual rickettsias, there is a clear unstained halo of variable width; recognition of this halo is frequently of great assistance in identifying the organisms though it must be stated that whenever a typical rickettsia is seen there is seldom any doubt as to the identity.

Rickettsias have been found more constantly in preparations from the testis but they have been present in peritoneum and splee surface films.

Strains " Appleton ", " Robertson " and Fièvre boutonneuse.—As regards the frequency of the organisms, their localization and morphology, these strains were identical with strain " Hare " in all respects.

Strain Rat Typhus.—In striking contrast to the scarcity of the organisms found in the exudate of guinea-pigs infected with strain " Hare ", with the rat typhus strain, infected cells were frequently found in enormous numbers. Although the best preparations have been obtained during the early stages of the scrotal reaction infected cells have been found up to the 7th and 8th day. The typical location is intracytoplasmic although numerous organisms were extracellular, again undoubtedly due to mechanical factors. Only one type of cell has been found parasitized, viz., serosa cells, and rickettsias have not been found in monocytes or neutrophiles except under conditions certainly related to mechanical and traumatic factors. Within the serosa cells the rickettsias show a decided tendency to be aggregated in clusters or colonies; this

tendency is exemplified in plate 2, fig. 3, which illustrates a cell containing a limited number of organisms yet the clumping is well-defined. Typically an infected cell is crammed with rickettsias (plate 2, fig. 5) so much so that the outline of the cell and the nucleus is distorted. Such cells may be picked out easily under low magnification since the distended dark blue cytoplasm is conspicuous. The rickettsias stain a delicate bluish purple and morphologically give the impression of being longer and finer than rickettsias of strain " Hare ". In the early stages of the reaction when individual cells contain only a limited number of parasites there is a definite tendency to chain formation so that one may pick up cells containing a thread-like network of rickettsias. As proliferation advances the organisms tend to become smaller so that when they are most numerous they take on a diplococcal or cocco-bacillary form. The morphology of individual organisms can be studied best by observing the extracellular forms. Usually these are in pairs consisting of two delicate rods separated by an unstained space of variable size, though frequently there is no intervening space so that a single bacillary form is found. The ends are usually rounded though they may be lanceolate. In the majority of cases the pairs are in a straight line but they may be bent at any angle and occasionally crescent forms are seen. Never have any intranuclear rickettsias been observed although particular attention has been paid to this point.

Again rickettsias were found in greatest numbers and with greatest regularity in preparations from the tunica vaginalis of the testes; usually they could be demonstrated on the testes before being seen in films from the peritoneum or spleen surface. It may be stated generally that the order of their appearance was first on the testes, then on the peritoneum and then on the spleen and that they disappeared in the same rotation.

Cultivation on the Chorio-allantoic Membrane of the Chick Embryo.

Apart entirely from reports on the application of tissue culture methods to the cultivation of the rickettsias of the typhus—Rocky Mountain spotted fever groups of disease, the literature contains several references to the use of the chorio-allantoic membrane of the developing chick embryo as a culture medium. Zia (1934) reported the cultivation of European and Mexican typhus rickettsias through 3 generations and came to the conclusion that there was very little hope of applying the method to mass production for vaccine purposes. Da Cunha (1934) cultivated the Sao Paulo typhus rickettsia, demonstrated the presence of organisms in membranes of the second generation, and showed their infectivity for guinea-pigs. Bengtson and Dyer (1935) cultivated the virus of Rocky Mountain spotted fever on the chorio-allantois through 20 passages without diminution in virulence for either the embryo or guinea-pig and described the lesions produced in the membrane and the morphology of the rickettsias. On the other hand, Pijper and Crocker (1938), failed to obtain any multiplication of tick-bite fever rickettsias on egg membranes. They point out that no serial transfers from egg to egg were made, and it will be seen below that this omission was probably

the cause of their failure. The technique of Barykine, Kompanesy, Botcharowa and Baver (1938) who recommend injecting infective material into the yolk sac of 3 to 4 day old embryos, has not been investigated up to the present time. In the present report details are given of the cultivation of 4 strains of rickettsias.

Technique.—The technique of inoculating the eggs was that described for similar studies on the propagation of the neurotropic virus of horsesickness (Alexander 1938). The inoculum used for seeding was a saline emulsion of scrapings from the tunica vaginalis of guinea-pigs sacrificed at a stage slightly before the height of the scrotal reaction. In some cases this reaction was marked, in other cases barely detectable, and on two occasions the inoculum was prepared at a time when a reaction, had it occurred, could reasonably have been anticipated; yet in every instance a successful series of cultures was initiated without undue difficulty. The eggs containing 8 to 10 day old embryos were incubated at approximately 33° C. (91·4° F.) for from 4 to 6 days. A lower temperature could not be used because, particularly in the summer months, the laboratory temperature rises to 33° C. or higher. An incubation temperature lower than that usually employed for bacteriological purposes was used because of the experience of other workers on cultivation by in vitro methods and also because it was early apparent that better results were obtained. The low temperature may account for some mortality of the embryos but the procedure was entirely practical. Material for transfers was prepared by emulsifying pieces of membrane about 1·5 cm. square selected from two eggs from the site of inoculation. The pieces of membrane were minced finely with scissors, then ground up with the roughened end of a glass rod without the addition of sand and before adding 25 c.c. of sterile saline. The emulsion was not clarified by centrifugation because the particles of membrane quickly disperse either to the surface of the fluid or the bottom of the tube so that it was possible to remove turbid fluid free from any conspicuous particulate matter from the middle portion. The sterility of each inoculum was tested by seeding 0·5 c.c. on to ordinary agar and incubating for 2 days at 37° C. Tests for rickettsial infectivity were carried out by inoculating guinea-pigs intraperitoneally. Usually 6 eggs were seeded with each inoculum (dose 0·2 c.c.) at every transfer and it was found essential to run a series in duplicate to minimize the risk of total loss from bacterial contamination. It is necessary to emphasize that it is far easier to carry on a series of cultures where the embryo itself is used as a source of virus. A slight bacterial contamination in the majority of cases appears to remain localized on the membrane, absorption of the virus is not interfered with and a sterile embryo emulsion is the outcome; on the other hand, it is apparent that the bacteria contained in a contaminated seeding fluid find the membrane a suitable medium (for propagation) and in this work many cultures had to be abandoned.

The technique of preparation of impression smears of the membranes was that described by Bengtson and Dyer (1935). A portion of the membrane at the site of the inoculation was excised, placed ectodermal side down on a clean glass slide, and covered with a strip of blotting paper over which was placed another slide. The

two slides were clamped together by means of a stout binder clip and gently flamed. The blotting paper was then removed leaving an impression of ectodermal cells behind and a second glass slide was placed in apposition to the remnant of membrane adhering to the blotting paper. In this way it was possible to make 3 preparations from each membrane.

The Lesion on the Membrane.—Since the gross macroscopical lesion appeared to be identical in the case of all the strains of rickettsias cultivated, a single description will suffice. It was not possible to follow the development of the lesion in any particular egg as the window method of seeding was not used but during the course of the work eggs were opened at different stages so that an accurate conception of the typical picture has been obtained.

In the early stage of infection (2nd day) the membranes lose their smooth, glistening, and transparent appearance and show the presence of a variable number of opaque areas, pin-point in size. It was found that the number of opaque spots was quite inconstant in different eggs seeded at the same time with the same emulsion and opened simultaneously, although it is admitted that had considerably greater care been taken to prepare a uniformly disperse inoculum, this discrepancy might not have occurred. However, the impression has been gained that in spite of refinement of technique, it would be exceedingly difficult to obtain uniform and constant multiplication of rickettsias in different eggs, and consequently the number of lesions per membrane would be variable. Later (3rd and 4th day), the opaque spots have increased in size but there appears to be little uniformity, minute pin points and areas of opacity being evident at one and the same time. Still later (the 4th and 5th day) the opacity has become confluent so that after removing the egg shell and shell membrane it is not possible to see the structure below. At this stage the membrane is thick (fully 2 mm.), moist and oedematous and when removed may have the appearance of a piece of greyish-white opaque jelly. In about 10 per cent. of cases it was noticed that a white amorphous powdery material accumulated on the ectodermal surface; its nature or significance is not known but it bore no connection with bacterial contamination and was not correlated with abundance of demonstrable rickettsias. After the death of the embryo the membrane takes on a dry, greyish, crinkled appearance.

Infection with the rickettsias studied destroys the embryo. In the case of the rat typhus strain approximately 40 per cent. of the embryos were dead on the 6th day after seeding, the majority were dead on the 7th day and it was rare for any to survive 8 days. It may be argued that at least the late deaths was the result of incubation at an unfavourably low temperature. Control eggs, however, seeded with either saline or non-infective emulsion frequently survived for longer than 8 days, although in some experiments there was a mortality of 30 per cent. on the 8th day. Further, in the case of the " Hare ", " Robertson " and *fièvre boutonneuse* strains, embryos commenced to die on the 4th day and it was rare for any to survive 6 days. Therefore it is apparent that in the case of these strains at least, the organisms and not the unfavourable hatching conditions were the cause of death.

It is necessary to mention that when initiating a series of egg cultures the results obtained with the first few generations are somewhat disappointing. In the majority of cases the control guinea-pigs inoculated with membrane emulsions comprising the first, second, or even the third generation either failed to react and later were found to be susceptible or showed an indefinite thermal or scrotal reaction and later were found to be immune or susceptible. Although the early generations might be non-infective for guinea-pigs and rickettsias not demonstrable in the impression preparations, later generations of the same series might be virulent and yield smears showing many organisms. Therefore it was always necessary to continue subinoculations for at least 3 generations before deciding if the attempted cultivation had been successful or not. After a strain had been "adapted" to eggs it was simple to alternate from guinea-pig to egg, the first generation on membranes always proving fully infective.

Morphology and Characteristics of the Rickettsias.

Strain "Hare".—Typical rickettsias were found in all generations from the 4th onwards, though the number of rickettsias in preparations from different eggs varied within very wide limits. Moreover, in many smears organisms were localized in definite small areas as if they had been present in isolated nodules.

Morphologically there was no difference between the organisms seen in membrane smears and those found in tunica smears from guinea-pigs except that the greater number present emphasized the extreme pleomorphism. No difficulty was ever experienced in identifying the bacillary forms but care has to be exercised in differentiating coccal, diplococcal, and cocco-bacillary forms from the granules of the immature polymorphonuclear cells which appear to aggregate at the site of multiplication. However, the rickettsias stain a delicate bluish purple and invariably are surrounded by a more or less distinct halo while cell granules stain reddish in colour and have a metallic lustre. In spite of these criteria many granules were seen about which there exists a definite doubt; the large organisms referred to by Bengtson and Dyer (1935) were encountered but no decided opinion as to their identity or significance can be expressed at present.

In some preparations rickettsias were exceedingly numerous (many hundreds per field) but this was the exception. Typically they were not particularly frequent and a good preparation was considered one that contained 5 or 6 infected cells per field over a number of separate areas each limited to about 20 fields in extent. In at least 20 per cent. of preparations it was not possible to demonstrate rickettsias at all, yet an emulsion would prove highly infective for guinea-pigs. The reason for the variation in the number of organisms in smears from different membranes is not known; it is unlikely to be associated with the somewhat crude method of preparing the impression.

The majority of rickettsias are intracellular or are seen in the vicinity of a ruptured cell; on the other hand, in a good preparation, large numbers may be found scattered amongst intact cells. In the cytoplasm of infected cells they were rather more numerous than in

guinea-pig preparations (up to 100 per cell) but they were always scattered in a disorderly manner and there was no tendency for them to be aggregated into clumps (plate I, figs. 1 and 1A). However the important feature is that intranuclear forms were common (plate I, fig. 2A), although the intracytoplasmic forms were by far the more frequent. The number of rickettsias within the nucleus varied from a single individual to a crowded mass which distended and distorted the nucleus. Morphologically they did not differ from the extranuclear forms though it will be appreciated that recognition of the smaller forms was exceedingly difficult. Unless the staining is good and carried out at a pH not more acid than 7·4, identification within the nucleus is difficult and great care must be taken with the examination. Later the use of Pinkerton's stain greatly facilitated determination of the intranuclear habitat. When the number of intranuclear parasites is limited, a well-defined halo is frequently evident thus giving the appearance of a small bacillus lying in an unstained hole in the nuclear chromatin; small numbers may lie in apposition to one another in a manner suggesting division by binary fission; when large numbers are crowded together the halo is obscured and the general picture is that of bluish-purple rods lying between or embedded in the reddish chromatin. The only tendency to the formation of groups or clusters of rickettsias has been in the case of the intranuclear forms; these infected cells may be picked out under low magnification owing to the decided bluish colour of the nucleus. This intranuclear localization was a striking feature.

Three attempts have been made to determine the effect of long continued passage upon the virus but unfortunately the procedure has been abandoned on each occasion owing to bacterial contamination, once at the 9th, once at the 11th, and once at the 16th subculture. All generations from the 5th to the 16th were fully virulent for guinea-pigs and rickettsias were demonstrable in the impression smears. It is worthy of note that on many occasions an emulsion of contaminated membranes has produced the normal febrile and scrotal reaction on intraperitoneal injection into guinea-pigs, and that smears showed rickettsias intermingled with the easily recognizable cocci or bacilli. All attempts to separate rickettsias from contaminants by dilution methods either in the guinea-pig or on eggs have proved unsuccessful.

The reaction produced by membrane virus in guinea-pigs differed from that produced by infective guinea-pig brain in the course of routine passage only by the significantly shorter incubation period and the more constant incidence of a well marked scrotal reaction (practically 100 per cent. in sexually mature guinea-pigs). This is almost certainly due to the large number of infecting doses contained in the membrane emulsions; in serial dilution experiments to determine the infective titre, the guinea-pigs which received the higher dilutions reacted in a manner identical with that of the guinea-pig passage animals. On several occasions it was found that injection of a dilution representing 1 in 12,000 of the original membrane was infective. It should be mentioned that guinea-pigs which receive higher dilutions may show no clinical reaction yet on immunity test may or may not be found to be immune (subclinical or inapparent infection).

The effect of passage on eggs appeared to result in an increase of virulence for the embryo. In the early generations subculture could be delayed until the 6th day after seeding; later it became necessary to subculture on the 5th day and the reason why at least one of the late generation cultures was lost was that all embryos were found dead on the morning of the 5th day though they had appeared normally active on candling the previous afternoon.

Portions of membrane some distance from the site of inoculation, i.e., membrane not included in the artificial air sac, were found to be infective though the reaction produced indicated that a large quantity of virus was not present. Similarly an emulsion of an embryo produced a mild reaction without scrotal involvement in 2 guinea-pigs which were found to be immune 4 weeks later.

Strain "Robertson".—The behaviour of this strain on eggs was practically identical with strain "Hare". Morphologically the 2 rickettsias in impression smears are indistinguishable and some beautiful preparations demonstrating the intra-nuclear forms were obtained.

It has been shown in another publication (Part III, this journal) that this strain of rickettsia is maintained in guinea-pigs with some difficulty, that the reactions produced by infective brain material are sometimes indefinite with a low percentage involvement of the scrotum and testes, and that inapparent infections are frequent. On the other hand egg-membrane virus proved highly virulent for guinea-pigs, produced a well-marked febrile reaction with a short incubation period (3 days) and a high percentage of scrotal lesions (about 70 per cent. in mature guinea-pigs). A noteworthy feature was the rapidity with which the scrotal lesion developed. Slight redness, swelling and oedema only might be noticed in the morning and yet by midday or the early afternoon the reaction would be pronounced. If anything strain "Robertson" proved more virulent for the embryos than strain "Hare" so that sub-culture on the 4th day was necessary on many occasions.

Strain "Appleton".—This strain had been abandoned before the egg-membrane culture technique had been perfected.

Strain "Fièvre boutonneuse".—The behaviour of this strain was identical in all respects to strain "Robertson".

Strain Rat Typhus.—The murine strain of rickettsia was found to multiply prolifically on the chorio-allantoic membrane. The initiation of cultures appeared to be somewhat more difficult than in the case of other strains. On two occasions testicular washings which were shown to be highly infective for guinea-pigs failed to establish infection on eggs. Further, in successful series before rickettsias could be demonstrated and before control guinea-pigs showed well-defined reactions it was necessary to carry out at least three egg-to-egg transfers.

The morphology of the rickettsias did not differ from that seen in preparations from guinea-pig tests, but again the rather more numerous organisms emphasized the extreme pleomorphism. Coccal and diplo-coccal forms, some so small as to be just within the range of

visibility, were common but the bacillary forms were also numerous. A consideration of the photo-micrographs indicates the intracytoplasmic habitat but, what is more important, shows very clearly the formation of masses of innumerable intracellular organisms in marked contrast to the irregular scattering of the other strains. Further it is of the utmost importance to emphasize that in spite of a very careful search through hundreds of preparations intranuclear forms were never observed. Individual organisms were seen lying on top of a nucleus but in every case it was abundantly clear that the rickettsias had been superimposed mechanically.

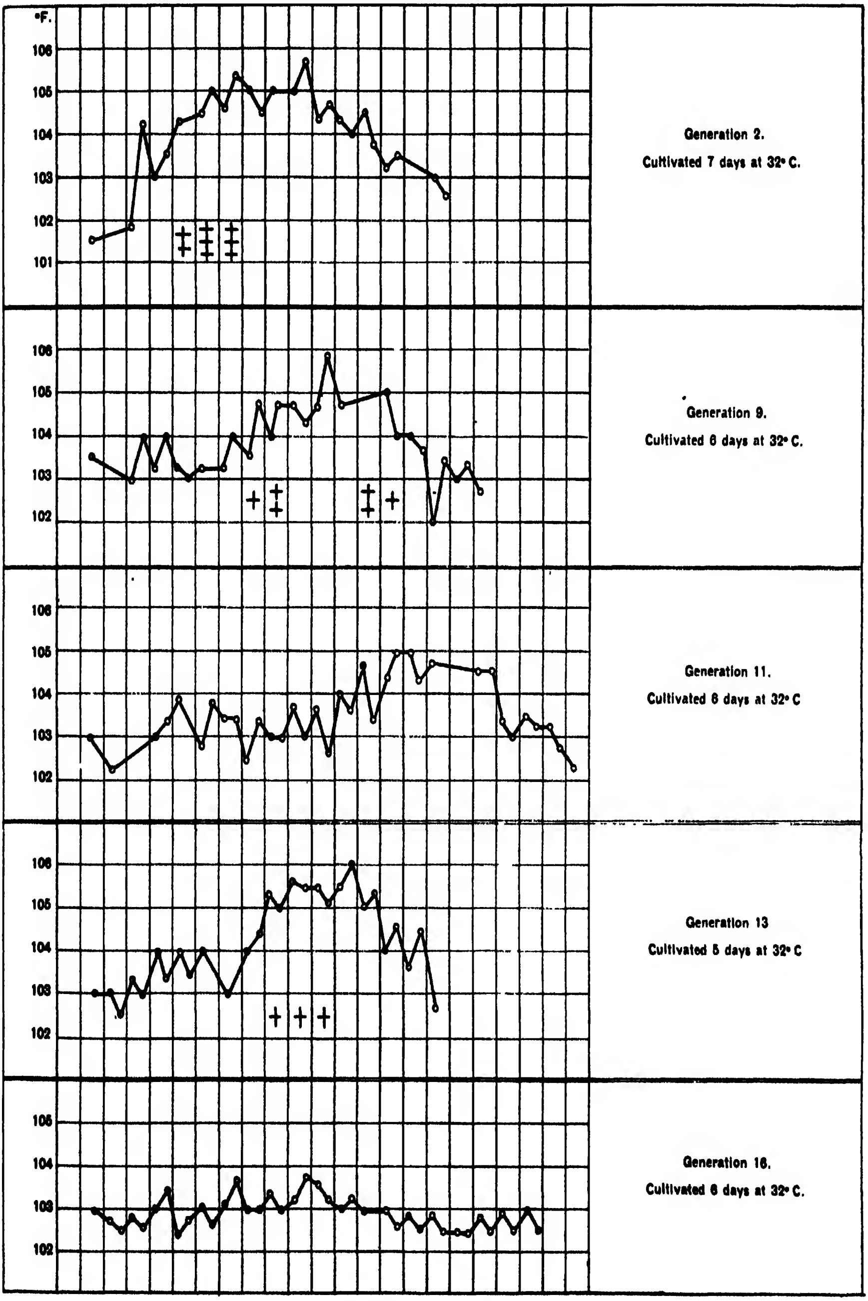
Four attempts were made to determine the effect of continued serial passage. A series of temperature curves is shown (Chart I) which illustrates the tendency to die out on repeated sub-culture. These charts indicate the reactions produced in guinea-pigs by a strain which, first having been adapted to the chorio-allantoic membrane, was then passed through one generation in guinea-pigs before being transferred back to eggs. A consideration of the charts shows that the second generation on membranes produced a severe reaction characterised by a short incubation period and a marked scrotal reaction. The 9th generation was infective but the incubation period was considerably lengthened and the degree of scrotal swelling was less. The 11th generation culture produced a slight febrile reaction which was delayed, and no scrotal lesion. While generation 13 was again infective, generation 16 was avirulent. Generation 18 has not been reproduced in the chart but it was non-infective. On immunity test applied 6 weeks after the test injection all guinea-pigs up to generation 13 were solidly immune; the guinea-pigs of generation 16 and 18 were fully susceptible. In addition to the loss of infectivity for guinea-pigs, impression smears up to generation 9 consistently showed the presence of many rickettsias, though in the latter passages there appeared to be a preponderance of minute coccal and cocco-bacillary forms. From the 10th sub-culture onwards rickettsias became progressively scarce and could not be found in passages 13 to 18 when the experiment was abandoned.

Out of 4 attempts at serial passage one was abandoned at generation 6 owing to bacterial contamination and, in the other three, infection died out after the 13th sub-culture. Sufficient work has not been carried out to determine with certainty whether this tendency to die out on serial passage through eggs is a definite characteristic of the murine strain or whether it represents either an inexplicable coincidence or some deficiency in technique which has not been appreciated. The point is being subjected to further investigation, but for the purposes of the experimetnal work involved in this study it has been found advisable never to continue a series for more than 9 generations, and then to continue with tunica washings after 1 passage through the guinea-pig. Moreover, for microscopic purposes, impression smears were made from cultures not less than generation 3 and not more than generation 9.

In connection with the effect of the rat typhus strain on the embryo itself it must be stated that it appeared to be less virulent than the other strains. Subcultures are usually carried out on the 6th day after seeding and survival of the embryos for more than 7 days was not uncommon.

CHART I.

Temperature Reactions in Guinea-pigs Produced by Egg-membrane Cultures of Rat Typhus.



+ – +++ = Degree of Scrotal Reaction.

Discussion.

Apart entirely from the demonstration that the chorio-allantoic membrane of the chick embryo constitutes a suitable medium for the propagation of rickettsias, the results of the experimental work detailed above bring out several points of great interest.

At first it was believed that the large numbers of organisms found in smears of infected membranes, indicated the application of the method not only to vaccine production, but also to the production of antigens for such purposes as agglutination, complement-fixation, and *in vitro* neutralization tests. It was early apparent however that multiplication was too irregular and too inconstant for these purposes. It has been shown that the rickettsias studied fall into two main groups:—

I. A group where the organisms in the guinea-pig are easily demonstrated as masses within the cytoplasm of serosa cells only. On artificial cultivation this tendency to intracytoplasmic aggregation persists but an intranuclear habitat is not assumed.

II. A group where the organisms in the guinea-pig are not only extremely rare but when found are sparsely distributed throughout the cytoplasm of both serosa cells and monocytes without any tendency to aggregation into clumps. On adaptation to multiplication on egg membranes these rickettsias, although present in much larger numbers, again are dispersed throughout the cellular cytoplasm but in addition also have an intranuclear habitat. Within the nuclei they may be present in aggregations of uncountable numbers of organisms. This group includes all the strains other than rat typhus.

Minor morphological differences appear to exist between the rickettsias of the two groups, e.g. as a rule those of rat typhus appear to be rather finer and more delicate in contrast to the rather more squat, plumper forms of the other group. These differences in morphology, however, cannot be regarded as of equal significance to the criteria enumerated above from the point of view of differentiation.

In conformity with the views of Pinkerton (1936) it would appear that some evidence has been brought forward to place the above group II [strains "Hare", "Appleton", "Robertson" (tick-bite fever) and fièvre boutonneuse] into his Rocky Mountain spotted fever group and group I (rat typhus) into his typhus group. It is obvious that even this broad classification could not be attempted without due consideration being paid to other essential properties of the virus strains so that a full discussion will be delayed until consideration of part III of these studies.

Summary.

1. The microscopic appearance of the scrotal exudate in guinea-pigs infected with each of five strains of rickettsia is described.

2. The technique of cultivation on the chorio-allantoic membrane of the chick embryo is described.

PLATE 1.

3. The morphology of the rickettsias as they appear in the scrotal exudate of the guinea-pig and on the chorio-allantois is described and compared.

Fig. 1.

Fig. 2.

Fig. 1.—Strain "Hare" guinea-pig tunica preparation.

Fig. 2.—Strain "Hare". Chorio-allantoic membrane preparation.

Fig. 1A.

Fig. 2A.

Camera lucida drawings of above.

PLATE II.

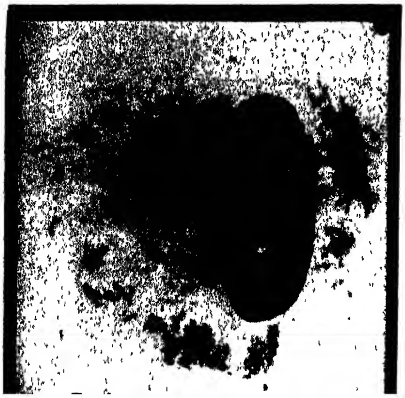


Fig. 3.

Fig. 4.

Fig. 3.—Rat Typhus. Guinea-pig tunica. Small number of intracytoplasmic organisms showing tendency to clumping.

Fig. 4.—Rat Typhus. Guinea-pig tunica. Intracytoplasmic mass of rickettsias.

Fig. 5.

Fig. 6.

Fig. 5.—Rat Typhus. Membrane culture showing mass of intracytoplasmic rickettsias.

Fig. 6.—Rat Typhus. Membrane culture showing mass of coccal and cocco-bacillary forms Cell ruptured.

PLATE II (*continued*).

Fig. 7.—Camera lucida drawing of cell similar to Fig. 5.

Fig. 8.—Camera lucida drawing of portion of Fig. 6.

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Studies of the Rickettsias of the Typhus-Rocky-Mountain-Spotted-Fever Group in South Africa.

III.—The Disease in the Experimental Animal. Cross-Immunity Tests.

By

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IN this section, we shall deal with the effect of the various typhus strains on the guinea-pig, rabbit, rat, mouse, dog, sheep, and ox, and with cross-immunity tests carried out in the guinea-pig and in the sheep.

RAT TYPHUS.

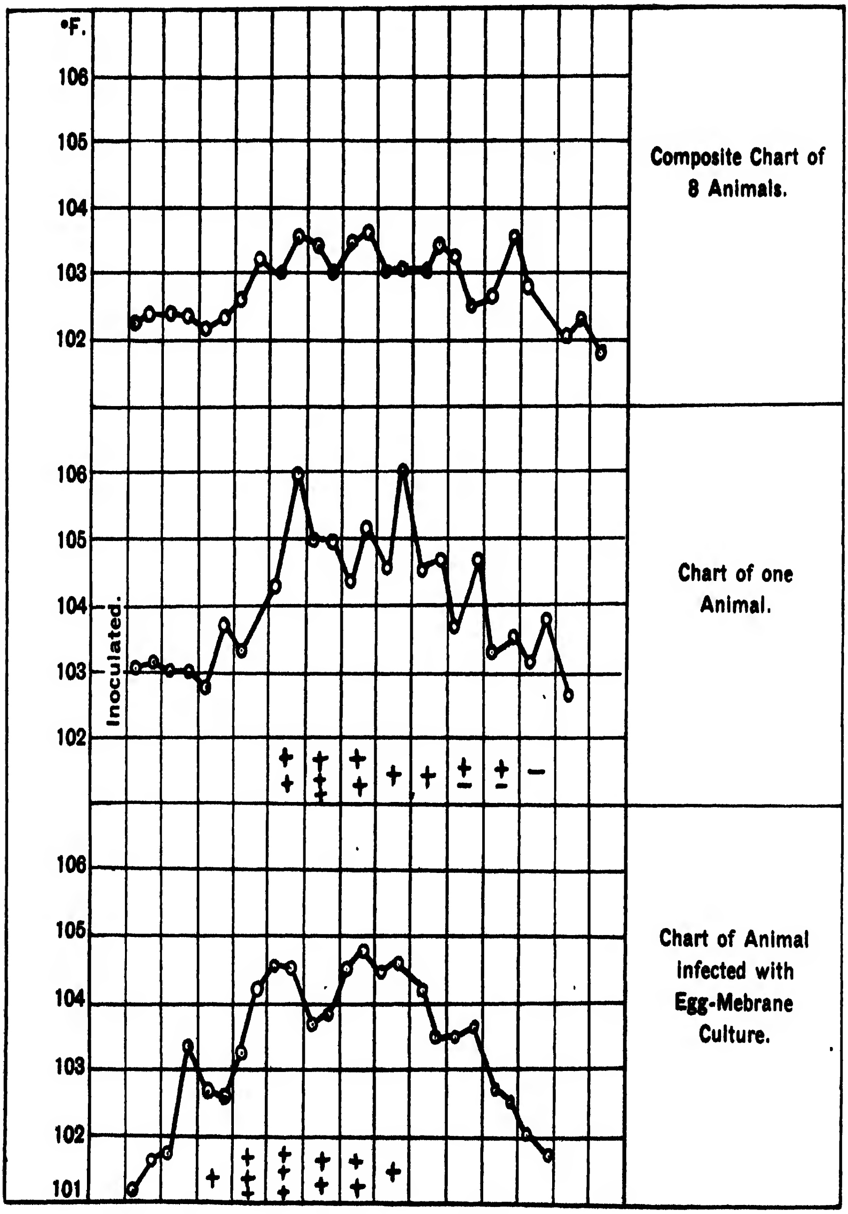
In the Guinea-pig.

No difficulty has been experienced in maintaining this rat typhus strain in guinea-pigs by brain to peritoneum passage every 9th or 10th day.

The type of reaction produced in the guinea-pig did not differ from that described by most workers in the typhus field. The first rise in temperature took place usually on the third, fourth or fifth day after infection and, generally, the disease (as manifested by the temperature reaction) was of a more protracted nature than that caused by "Robertson", "Hare" or *fièvre boutonneuse*. In common with our experience with the other four virus strains employed, a "saddle-back" type of curve was common; the initial rise was maintained for one or two days and was followed by a lower temperature during the next day or two and then by a secondary rise which returned to normal by lysis. Chart 1 shows a composite temperature curve of eight infected guinea-pigs, a typical curve of one animal and the reaction produced by an egg-membrane culture. The composite chart, although reproducing the

CHART 1.

Rat typhus in guinea-pigs.



+ - +++ = Degree of Scrotal Reaction.

general trend of the reaction, is not typical; each animal does not react to the same degree at the same time, so that a flattening of the curve is produced. This point must be kept in mind when composite charts are read. No deaths directly attributable to rat typhus occurred among the hundreds of guinea-pigs that received the virus (brain, tunica or egg-membrane) and, except for the temperature and scrotal reactions, the animals looked healthy.

The Scrotal Lesion.

In sexually mature, male guinea-pigs, i.e. in those with descended, palpable testes, a tumefaction of the scrotum was an almost constant phenomenon. Of 89 males, 84 (94·4 per cent.) showed this reaction and if adults only had been inoculated, this percentage would have been almost one hundred. After 50 sub-inoculations (brain to peritoneum) this reaction occurred as regularly and as strongly as in the first passage carried out by us. The day of the appearance of the swelling in 38 guinea-pigs was as follows:—

Day on which swelling was first noticed	4	5	6	7	8	9	10
Number of guinea-pigs involved	5	15	7	4	5	1	1

The majority of the reactions were definite by the fifth day after infection; those appearing for the first time on or after the seventh day were atypical and were associated with a lengthened (temperature) incubation period. The rapidity with which a scrotum could swell was remarkable; often only a few hours elapsed between a doubtful and a " + + + " reaction. The maximum enlargement, once reached, was maintained for from two to four days; thereafter reduction occurred, the return to apparent normality occupying from three to seven days.

In character, the scrotal reaction in rat-typhus-infected guinea-pigs differed somewhat from that produced by the other strains. At the height of the reaction, the scrotum was tense, hard, and firm and lacked that fluctuating, oedematous feeling of the swelling produced by " Hare " or " Robertson ". As a rule, it was not possible to force the testicles into the abdominal cavity and, even at post-mortem examination, it was frequently impossible to draw them out of the scrotum. Actually, on many occasions, it was necessary to dissect them out in order to make scrapings from the tunica. The testicles themselves showed distension and ramiform injection of the blood vessels, petechiae, even ecchymoses up to 3 mm. in diameter, and, not uncommonly, small haemorrhages in the polar fat. An exudate, which was fairly copious and fluid in the early stages of the reaction, later thickened to form fibrinous sheets that covered nearly the whole testicle. These sheets, greyish-white in colour, could be scraped off and floated intact in saline. The final outcome was usually *restitutio-ad-integrum* but, on several occasions, some thickening of the scrotum persisted that left the testicle hard and attached to the sac.

Post-mortem examination.—Apart from the scrotal lesion, the only other constant macroscopical change was a swelling of the spleen, often to two or three times its normal size. Not infrequently, a tenacious greyish-white deposit or even a pseudo-membrane covered the surface of the spleen and liver.

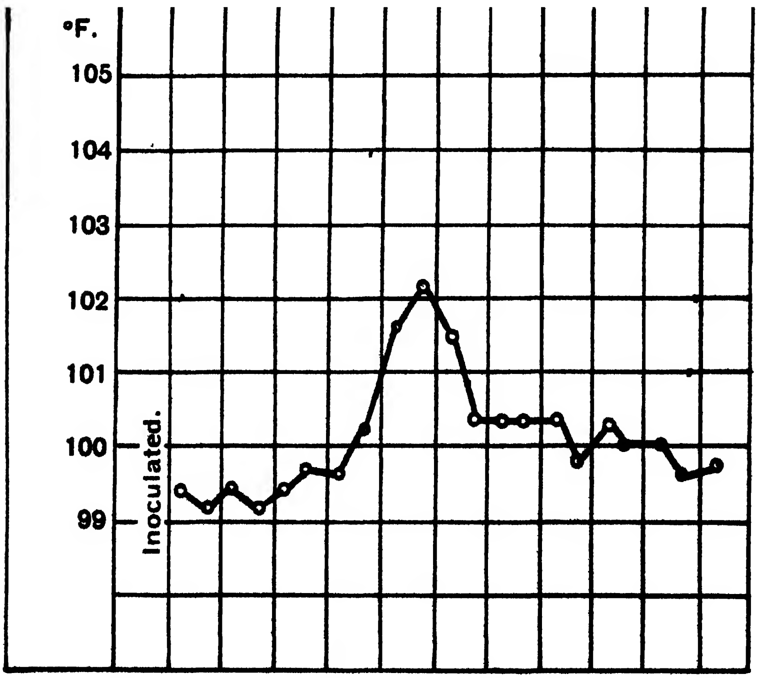
In the White Rat.

No difficulty was experienced in maintaining this strain in the rat; 50 animals were used, 12 subinoculations were made and the experiment was terminated at this point. The brain of a reacting guinea-pig was the first inoculum and thereafter the brain of a rat, taken on the 8th to the 10th day after infection. At each passage, a portion of the rat brain was also inoculated into two indicating guinea-pigs. Unless a definite, easily recognizable reaction was noted, the guinea-pigs were retained for one month to undergo an immunity test.

Most rats showed a definite thermal rise that began on the 4th or 5th day (102° F. to 103° F.) and that was maintained for from two to four days, but at no time was a scrotal swelling observed. Chart 2 is a composite temperature curve of 10 infected rats.

CHART 2.

Rat typhus in rats. Composite chart of 10 rats.



Apart from the temperature reaction, many rats were visibly affected by the virus; some were poor, thin or even emaciated, and 8 died. This debilitating effect was most marked in those animals which received benzol and olive oil subcutaneously in addition to the virus inoculation (see Zinsser and Castaneda, 1930). In all rats, the chief post-mortem lesion was an enlargement of the spleen which was sometimes three to five times the size of that of the normal animal. In smears taken from the surface of such an enlarged spleen, rickettsias were plentiful and in one preparation there were dozens of infected cells per microscopic field.

In the Mouse.

The disease was passed (brain to peritoneum at 8 to 9 day intervals) through 3 lots of mice only, the 4th and subsequent passages being negative. The mice showed no symptoms or macroscopic post-mortem lesions and, as the temperature of normal mice can rise or fall several degrees in a few hours, infection could be judged only by the use of indicating guinea-pigs.

In the Dog.

Durand (1933) showed that murine typhus caused an inapparent disease in dogs, that the virus, in some instances, could be recovered in guinea-pigs from the blood, brain or spleen, and that the Weil-Felix reaction became positive (chiefly OX19). However, the disease could not be passaged from dog to dog. Combiesco and Angelesco (1933) also set up a "maladie inapparente" and 14 days later recovered the virus from the brain. However, to our knowledge, no indication has been given that the dog plays a rôle in the spread of typhus. Our results confirm this; in fact, we could do no more than demonstrate a survival and translocation of the virus.

Eight young dogs, obtained from a pound, were used. Dog 1 (1972) inoculated intraperitoneally with the brain of an infected guinea-pig, did not react. Guinea-pigs which received an intraperitoneal injection of the blood (3 c.c. to 4 c.c.) of this dog on the 4th, 7th, and 15th day after the attempted infection did not react and, tested later, were not immune.

Dog 2 (2089) was infected in the same way as dog 1, and, 9 days later, its brain was removed and a portion injected, intraperitoneally, into dog 3 (2093) and into guinea-pigs. None reacted. After 8 days, the brain of dog 3 was injected into dog 4 (2097) and into guinea-pigs without a reaction being produced. Finally no reaction occurred when the brain of dog 4 was inoculated into guinea-pigs.

In the 3rd experiment, 4 dogs (1967, 1969, 1970 and 2030) were infected with guinea-pig brain by the intraperitoneal route. No apparent reaction occurred. Table 1 summarizes the results of injecting the blood and the brain of these dogs into guinea-pigs.

TABLE 1.

The Infectivity for Guinea-pigs of the Blood and Brain of Rat-typhus-infected Dogs.

Inoculum.	Days after Infection.	From Dogs.	Result in Guinea-pigs.
Blood: 3–4 c.c. i.p.	4th	5, 6, 7, 8	Positive.
	6th	5, 6, 7, 8	Negative.
	10th	5, 6, 8	Negative.
	14th	5, 6, 8	Negative.
	19th	5, 6	Negative.
Brain: i.p.	7th	7	Positive.
	14th	8	Negative.
	19th	6	Negative.

The foregoing results show that the dog was not easily infected with typhus and that the disease could be carried on in it. Virus was demonstrable in the blood on the 4th but not on the 6th day after infection and in the brain on the 7th but not on the 14th day.

In the Sheep.

About half of the sheep used reacted with an elevation of temperature (see Chart 3) when infective guinea-pig brain was injected intravenously, and the disease could be carried on in sheep for one passage only, when blood, taken at the height of the thermal reaction, was the inoculum. The reaction of the recipient sheep was much milder than that of the donor. No symptom, other than the temperature, was noticed and in no instance did death occur. Attempts to infect guinea-pigs with the blood or brain of sheep taken at the height of the thermal reaction were unsuccessful.

In the Rabbit.

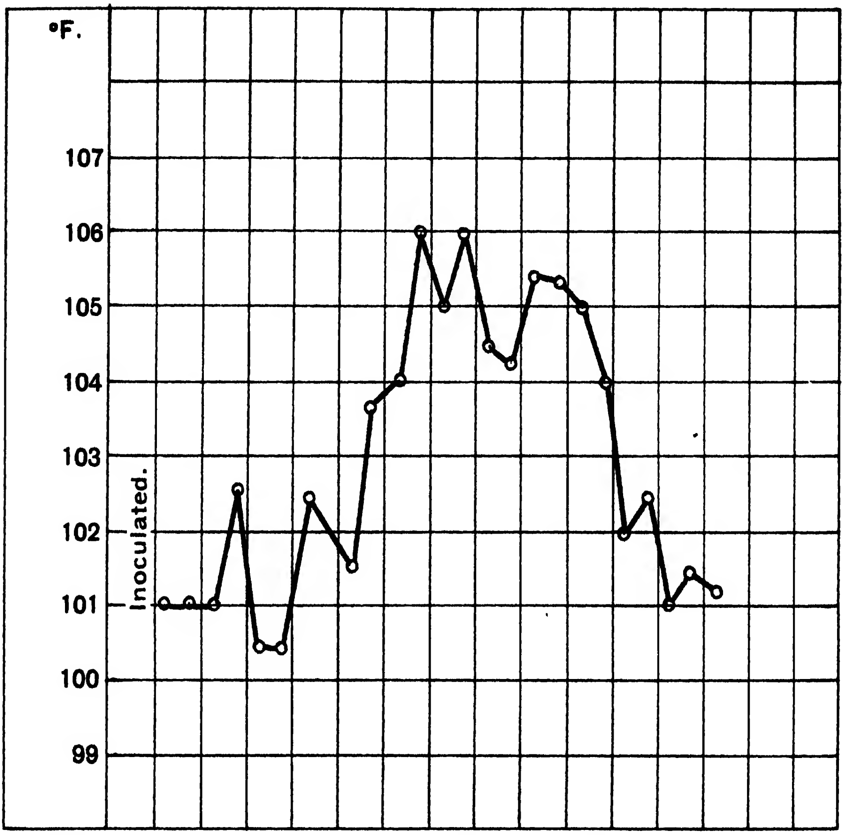
The effect of the rat typhus and the other virus strains on this animal will be discussed under " The Weil-Felix reaction ".

" HARE."

In the Guinea-pig.

Once established, no difficulty was experienced in maintaining the " Hare " strain in guinea-pigs (71 brain to peritoneum passages in 20 months at 8 to 10 day intervals). A composite temperature curve and a typical thermal reaction are reproduced in Chart 4. Usually the first rise was noted between the 4th and 6th days after inoculation, was maintained for from 4 to 7 days and slowly, with small fluctuations, returned to normal. It was unusual for a temperature of 106° F. to be recorded for more than 24 hours; generally the fluctuations were between 104° F. and 105° F. No symptoms, other than the elevated temperature and a scrotal swelling were observed.

CHART 3.
Rat typhus in sheep.



The scrotal lesion.—In the early passages, a scrotal swelling occurred very commonly in guinea-pigs; of 244 males, 211 (86·5 per cent.) showed this lesion and probably the percentage would have been higher if fully mature animals only had been used. In more recent passages, this reaction has been seen in only about 30 per cent. of cases, although the temperature curve has not varied in the slightest degree. The day of the appearance of the swelling in 34 guinea-pigs was as follows:—

Day on which swelling was first noted	4	5	6	7	10
Number of guinea-pigs involved	12	7	9	5	1 (late reactor).

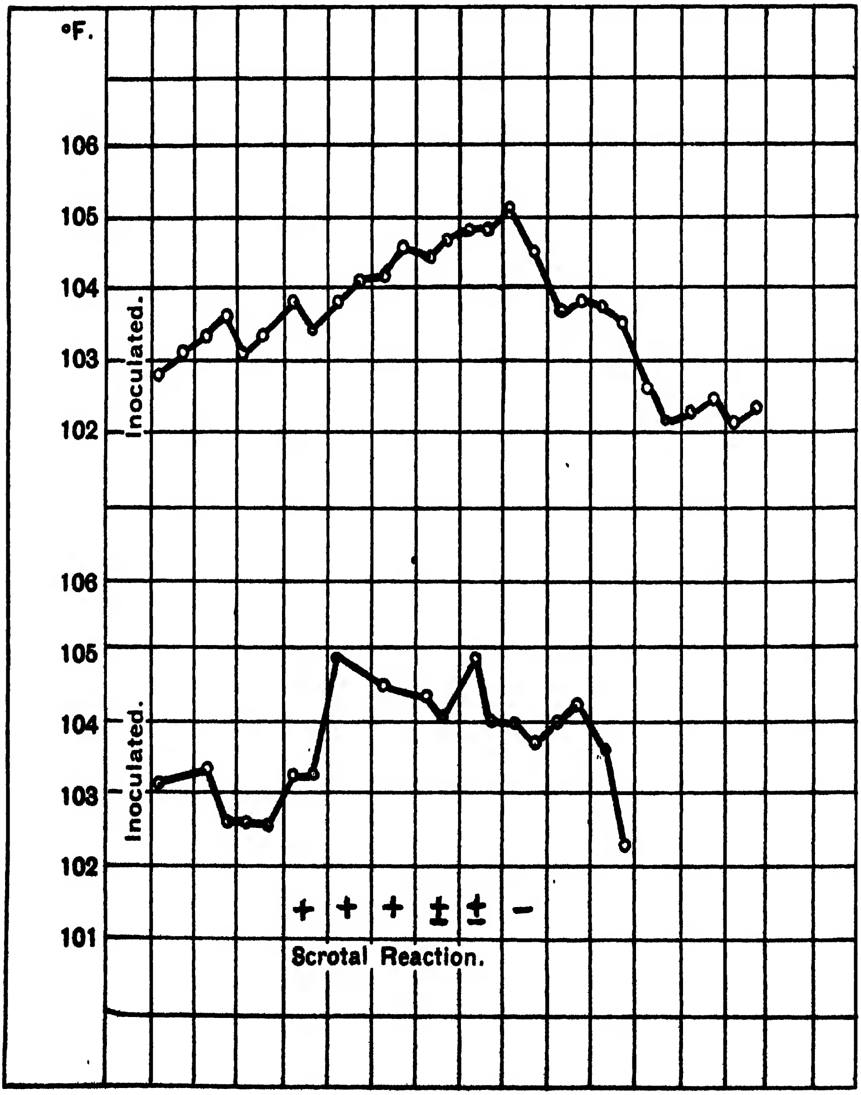
The enlarged scrotum felt soft and oedematous, and was not hard and firm as in rat-typhus-infected animals. Usually the testicle could be forced into the abdominal cavity and no thickening or hardness remained after the swelling had subsided. At post-mortem examination, in the early stage of the disease, the testes were hyperaemic and surrounded by a small quantity of greyish translucent exudate. Later, this hypermaemia became more marked,

but distinct haemorrhages in the testicle or polar fat were very rare. The exudate increased in amount, became viscid in consistency and whitish in colour and although flakes about 1 mm. in diameter could be scraped from the testicle, fibrinous sheets were not formed.

Post-mortem examination.—The remarks made under "rat typhus" apply to "Hare" with the reservation that the spleen was not usually so greatly enlarged (and occasionally was not apparently enlarged at all) and that a deposit on the spleen and liver was uncommon.

CHART 4.

"Hare" in guinea pigs. Composite chart of 8 guinea pigs (top). Chart of one animal (bottom).



In the White Rat.

Two attempts were made to adapt the "Hare" virus to rats, one involving 7 and the other 5 passages. As with the rat typhus rat passages, indicating guinea-pigs were inoculated at each sub-inoculation. Neither the rats nor the guinea-pigs reacted; the latter were later shown to be susceptible to a test inoculation of virulent brain.

In the Mouse.

No success attended two attempts to infect this animal. The brains of the mice of even the first generation (removed 9 days after the intraperitoneal inoculation of virulent guinea-pig brain) failed to cause a reaction in indicating guinea-pigs. Subsequent passages were also negative.

In the Dog.

One experiment only was carried out in this animal and although infection was not produced, we do not feel that the result is of great value. The pups, reared in tick-free surroundings, received infective guinea-pig brain intraperitoneally and showed no thermal or other reaction. Their pooled blood, taken at different times between the 2nd and 14th day after the injection did not infect guinea-pigs and these animals were not immune at a subsequent immunity test. However, a considerable number of the guinea-pigs died within one to four days after the blood injection and most of the remainder had severe temperature reactions beginning within 24 hours of the test. It has been our experience that a guinea-pig which has a non-specific temperature rise of this type does not develop typhus and, when tested later, is not immune. The lack of dogs, reared in tick-free surroundings, (and in work of this kind, it is essential to be certain of this point) has prevented our repeating the experiment.

In the Sheep.

What has been said about rat typhus in sheep applies also to "Hare".

In the Ox.

A bovine that received the tunica scrapings of a "Hare" infected guinea-pig intravenously did not react.

In the Rabbit.

See "Rat typhus".

"APPLETON".

In the Guinea-pig.

From the start difficulty was encountered in carrying this strain in guinea-pigs. A sustained rise in temperature (104° F. and above) occurred in about 30 per cent. of animals and a scrotal swelling was infrequent (about 10 per cent.). In an attempt to "acclimatize" the virus to guinea-pigs, we fed them on a vitamin-deficient diet

(autoclaved oats). This procedure proved to be disadvantageous because not only was the reaction no better but the guinea-pigs became thin and some died.

The Scrotal Lesion.

Although a scrotal swelling was uncommon, it occurred more frequently in the guinea-pigs of the earlier than of the later passages. The day of its appearance in 29 males was as follows:—

Day on which swelling was first noticed	2	3	4	5	6	7	8	9
Number of guinea-pigs involved	1	6	8	1	7	4	1	1

In character, the scrotal swelling produced by the Appleton strain was similar to that caused by " Hare ".

The post-mortem lesions were the same as those noted under " Hare ".

At the time when we discontinued the passage of this strain (65th guinea-pig passage) we had no experience in the use of the chick chorio-allantoic-membrane-method of cultivating typhus rickettsias. The use of this technique with a tick-bite fever (" Robertson ") and a *fièvre boutonneuse* strain indicates that an egg-membrane culture of " Appleton " rickettsias would probably have produced satisfactory reactions in guinea-pigs.

In the White Rat.

This strain was passed through eleven generations of rats (29 in all), at which stage the experiment was terminated. A period of 8 to 9 days was allowed to elapse between each passage. A rise in temperature to 102° F. or 102·5° F. (from normal of 99° F. to 100° F.) occurred on about the 3rd or the 4th day, was maintained for from 1½ to 3 days and thereafter fell fairly rapidly to normal. Indicating guinea-pigs inoculated with rat brain at each passage also reacted and were immune at a subsequent immunity test. Rats living on a deficient diet (autoclaved oats) and/or into which benzol and olive oil were injected subcutaneously, had no better reactions than those receiving a normal diet. The only apparent post-mortem lesion was an enlargement of the spleen. In Chart 5, a composite curve of the temperatures of 14 rats is given.

In the Dog.

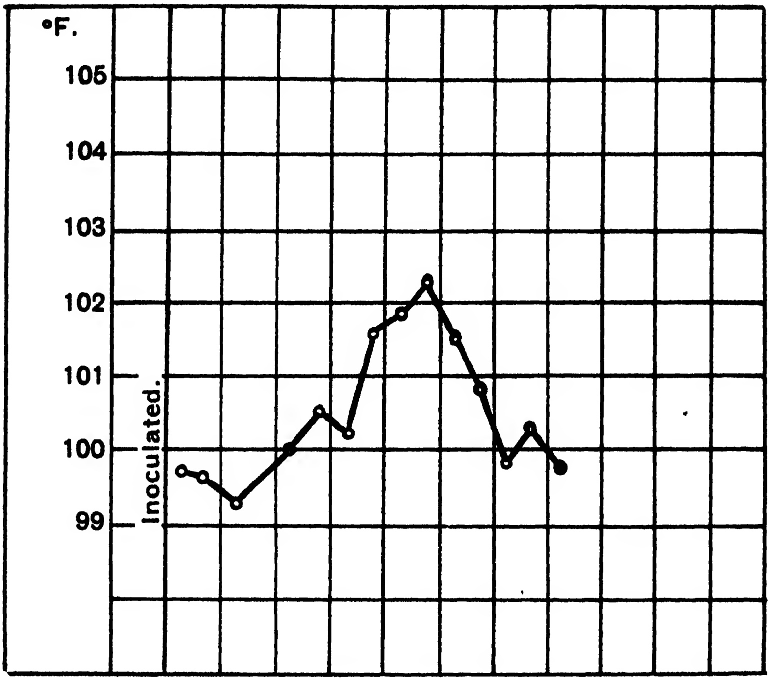
One dog (1935) inoculated intraperitoneally and another (1936) inoculated intravenously with the brain mush of two infected guinea-pigs showed no thermal or other reaction. As the dogs originated from a pound, we have no knowledge of their history; they may have been immune and, as no subinoculations were made from them into guinea-pigs, we do not know if they underwent an inapparent infection. However, it shauld be borne in mind that this strain originated from a dog, which was suffering, at least, from an inapparent infection.

In the Sheep.

See " Hare ".

CHART 5.

"Appleton" in rats. Composite chart of 4 rats.



"ROBERTSON."

In the Guinea-pig.

This, the only tick-bite fever strain of human origin with which we worked produced reactions (thermal and scrotal) only slightly better than those of "Appleton" when brain to peritoneum (8 to 10 day intervals) was the method of passage. As a routine measure we used three guinea-pigs per passage and as a rule one of these reacted fairly well, one poorly and one very slightly or not at all. The brain of such an apparently non-reacting animal taken on the 9th day after infection proved, on inoculation, to be just as virulent as that of a guinea-pig which had a good reaction. In general, the first thermal rise was late, very often not before the 6th or 7th day after infection. A temperature much exceeding 105° F. was seldom maintained for more than a day or two and generally the febrile period lasted for not more than 2 to 5 days and usually 3 to 4 days. Even in sexually mature male guinea-pigs, a scrotal swelling was uncommon, and occurred in not more than 10 per cent. of the animals. However, in spite of the poor reactions we succeeded in maintaining this tick-bite fever strain in guinea-pigs for more than a year (46 brain to peritoneum passages).

With the use of egg-membrane cultures we obtained much more definite reactions, both thermal and scrotal. In Chart 6 we reproduce a good temperature reaction produced by this means and include

a curve caused by the inoculation of brain. This chart, typical of many, shows that the infected egg-membrane caused an early temperature rise and an earlier-appearing scrotal lesion due, most probably, to the large number of rickettsias inoculated. This reaction was got in about 70 per cent. of sexually mature animals and followed much the same course as that of "Hare"; perhaps the only difference was that the scrotum seldom attained the same large dimensions. The lesions at post-mortem examination were the same as those given for "Hare".

In the White Rat.

The first rats were infected with egg-membrane culture and thereafter 10 successful subinoculations (at 8 to 10 day intervals) were made by brain to peritoneum passage. The reactions were almost superimposable on those obtained with the "Appleton" strain.

In the Rabbit.—See "Rat Typhus".

Fièvre Boutonneuse.

In the Guinea-pig.

We soon realised that it would be difficult to maintain this strain in guinea-pigs by brain to peritoneum passage. Even after 10 sub-inoculations (at 8 to 10 day intervals), there was no indication that the virus was adapting itself; reactions of the "Appleton" type were the rule. When a rise in temperature did occur, it was late in appearance (7th to 9th day), seldom rose much above 104·5° F. and was not maintained for more than 2 or 3 days. Of 31 inoculated animals, 15 had a thermal rise high enough to be considered significant and only one developed a scrotal swelling (small, appearing on the 7th day). Because we had two main objects in view—the study of the causative rickettsias and cross-immunity work—we decided to abandon brain to peritoneum passage and to resort to egg-membrane culture which had given such good results with another "weak" virus, that of "Robertson".

With egg-membrane cultures, no difficulty was experienced in producing good thermal and scrotal lesions. In Chart 7 two typical reactions are given. It will be noticed that the temperature rise occurred early and although it was not maintained for long, it was definite. The scrotal swelling, which occurred in about 60 per cent. of the male animals, usually appeared early and could not be distinguished from that of "Hare". The post-mortem picture was similar to that recorded for "Hare".

Summary of Reactions in Animals.

The results just presented show that rat typhus could be maintained, without any difficulty, in the guinea-pig and rat, and that in the former animal it nearly always caused a scrotal swelling. It could be passaged three times only in the mouse and not at all in

the dog. The sheep proved susceptible when inoculum was virulent guinea-pig brain, but the virus could not be passed serially through this animal. The single ox used proved to be insusceptible.

"Hare", alone of the other four viruses, was easily passaged in guinea-pigs; it caused readable temperature reactions and a scrotal swelling in a large percentage of cases. It did not infect the rat and the reactions in sheep were the same as those produced by rat typhus.

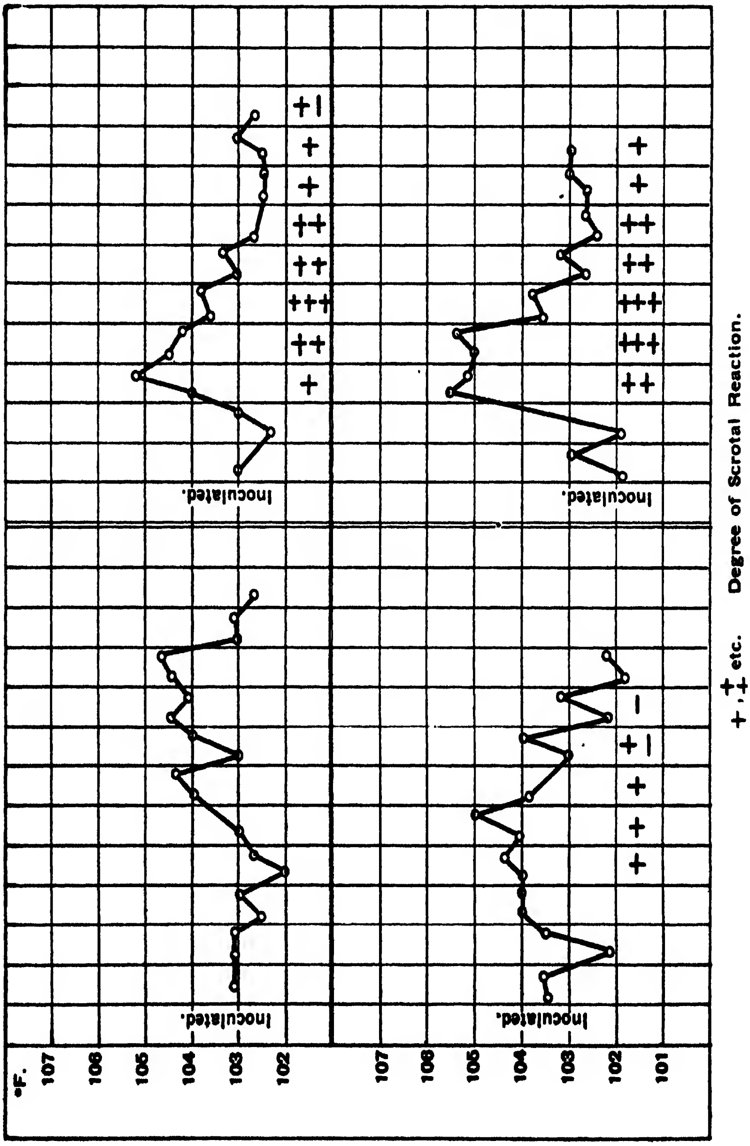


CHART 6.

"Robertson" in guinea-pigs. Chart of one animal (brain inoculum) (top). Chart of one animal (egg-membrane inoculum) (bottom).

CHART 7.

Fièvre boutonneuse charts of 2 guinea-pigs infected with egg membrane.

" Appleton " and " Robertson " and *fièvre boutonneuse* occasioned great difficulty when attempts were made to maintain them in guinea-pigs by the brain to peritoneum method of passage. However, when infected egg-membrane (in the case of " Robertson " and *fièvre boutonneuse*) was the inoculum, good temperature and scrotal reactions were obtained. Both " Appleton " and " Robertson ", although, as will be shown later, belonging to the same group as " Hare ", were able to infect the rat.

THE WEIL-FELIX REACTION.

In the Rabbit.

Rabbits were inoculated intraperitoneally with virulent guinea-pig brain (rat typhus and " Hare ") or with egg-membrane culture (" Robertson ") in an attempt to produce agglutinins to a proteus X strain. None reacted as the direct result of the inoculation and the serum of none, taken prior to the infection, agglutinated proteus OX2, OX19 or OXK at a dilution of 1:20 or higher. In carrying out the tests, living saline suspensions of non-motile OX2, OX19 and OXK were used. The cultures were grown according to the instructions sent out by Dr. Felix and every endeavour was made to prevent motile " H " variants appearing. This was never necessary with OX2, only very occasionally with OX19 and quite frequently with OXK. The tubes were incubated at 37° C. for four hours and left overnight at room temperature before being read.

Table 2 summarises the results of agglutination tests with serum taken at different times after infection.

A Weil-Felix reaction was obtained with the sera of 10 rabbits infected with rat typhus; in no instance was proteus OX2 agglutinated; OX19 was agglutinated by all and one case (rabbit 6) both OX19 and OXK were agglutinated. The sera of two of nine rabbits, inoculated with " Hare ", became positive; that of rabbit 14 agglutinated OX2, did not affect OXK and produced a doubtful reaction with OX19 and that of rabbit 15 agglutinated OX19 only.

The serum of one of seven rabbits inoculated with " Robertson " caused a doubtful agglutination of OX19, at a dilution of 1:20; the sera of the remaining six animals were negative.

In the White Rat.

The sera of 7 rats infected with rat typhus, and taken 8 to 36 days after the inoculation were tested with the 3 proteus X strains. Two were positive, both for OX19. That of one rat (27 days after infection) was postive at a dilution of 1:160 and that of the other (16 days after infection) at 1:40.

In the Guinea-pig.

In an attempt to isolate a specific proteus from guinea-pigs infected with rat typhus, " Hare " or " Appleton ", we cultured the intestinal contents of 26 of them and from 6 obtained a swarming proteus, the " O " antigens of which were not serologically related

TABLE 2.

WEIL-FELIX REACTION IN RABBITS.

Typhus Strain.	Proteus Strain.	Rabbit. 1			2		3		4			5		6	7		8		9	10	
		Days after Infection.																			
		11	20	38	11	17	11	17	12	15	25	10	23	20	9	13	9	13	11	15	32
Rat Typhus......	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	19	?20	—	—	160	160	320	40	160	80	20	?20	—	160	20	40	20	40	160	20	—
	K.	—	—	—	—	—	—	—	—	—	—	?20	—	80	—	—	—	—	—	—	—

Typhus Strain.	Proteus Strain.	Rabbit. 11	12	13	14		15		16			17		18		19	
		Days after Infection.															
		9	10	9	9	17	9	15	9	15	28	12	32	9	27	7	23
"Hare".........	2	—	—	—	—	80	—	—	—	—	—	—	—	—	—	—	—
	19	—	—	—	—	?20	—	80	—	—	—	—	—	—	—	—	—
	K.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Typhus Strain.	Proteus Strain.	Rabbit. 20		21	22		23		24		25		26
		Days after Infection.											
		9	17	9	10	16	10	16	10	14	10	16	14
"Robertson"....	2	—	—	—	—	—	—	—	—	—	—	—	—
	19	—	—	—	?20	—	—	—	—	—	—	—	—
	K.	—	—	—	—	—	—	—	—	—	—	—	—

— No agglutination at 1 : 20 serum dilution; 20, 40, etc. = reciprocal of the highest dilution of serum which caused agglutination.

to those of OX2, OX19 or OXK. It would appear that a proteus infection of the gut of guinea-pigs is not uncommon, because, even with the use of only a small number of animals, we obtained a 25 per cent. infection rate.

Giraud and Tannenbaum (1937,[1&2]) state that guinea-pigs do not develop agglutinins for proteus X during or after a typhus infection because they do not harbour banal proteus in their intestines; if this microbe is implanted in their alimentary canal by the oral and rectal routes and a typhus infection then set up, the Weil-Felix reaction develops.

To ascertain if proteus X agglutinins would develop if a banal proteus was implanted in the gut of guinea-pigs, we repeated Giraud and Tannenbaum's work. A heavy suspension of a swarming proteus [(R5H6), isolated from the small intestine of a rat and not serologically related to the " O " antigen of OX2, OX19 or OXK)] was administered to 6 guinea-pigs (1·0 c.c. fed through stomach tube and 1·0 c.c. per rectum) on 30.10.37 and thereafter, for 15 days, 10 c.c. was added to the food and 1·0 c.c. given per rectum. On the 16th day each animal received 10·0 c.c. per os and 1·0 c.c. per rectum. Three of these animals and three fresh untreated controls were infected, intraperitoneally, with typhus and three proteus-infected guinea-pigs left uninfected with typhus to see the effect of the proteus alone on agglutinin production. A Weil-Felix test, using living suspensions of OX2, OX19, OXK and R5H6, was put up with the serum of each animal on the day before the first proteus feeding, on the day of the infective inoculation and thereafter weekly for 5 weeks.

The results did not confirm those of Giraud and Tannenbaum. The serum of no animal, taken prior to the infection by proteus and/or rat typhus, agglutinated any of the four proteus strains used; those which were infected with rat typhus only remained negative throughout the experiment. The sera of the remaining 6 guinea-pigs (proteus alone and proteus plus typhus) at no time agglutinated OX2, OX19 or OXK, but all were positive for R5H6 (" H " agglutination) 17 days after the first proteus feeding and remained so for a further 4 to 5 weeks. The highest serum titre was 1:320.

Attempts to Isolate a Proteus from Guinea-pigs,

The brain, heart-blood, urine, tunica vaginalis, liver, and spleen of 238 guinea-pigs, infected with " Hare ", " Appleton " or rat typhus were inoculated into broth and on to nutrient agar and observed for 14 days at 37° C. About 1·0 c.c. of blood was deposited in a sterile tube and the serum, after separating, was removed and replaced with about 15 c.c. of broth. Small portions of brain, liver, tunica, and spleen were placed in broth, and about 1·0 c.c. of urine was cultured in the same medium. The agar cultures were made by smearing slopes heavily with the blood or urine or the organ mush. Any Gram-negative bacilli which grew were isolated in pure culture and tested against sera prepared against OX2, OX19, OXK and against the sera of sheep recovered from " Hare ", " Appleton "

or rat typhus. Eighteen such organisms were isolated—2 from the brain and 1 from the urine of "Hare" guinea-pigs, 1 from the brain, 1 from the urine and 1 from the liver of "Appleton" guinea-pigs, and 6 from the brain, 3 from the blood and 1 each from the urine, liver, and tunica of rat typhus animals. None were agglutinated by any of the sera used.

These results are in agreement with those of most workers who have tried to isolate a specific proteus X from guinea-pigs infected with one or other of the typhus strains.

The Duration of Infectivity of the Brain, Blood, and Tunica vaginalis of infected Guinea-pigs.

Guinea-pigs were infected by the intraperitoneal inoculation of virulent brain. At intervals after infection, the infectivity of the pooled citrated blood (3 c.c. to 4 c.c. intraperitoneally) of 4 to 6 of them was tested, and the infectivity of the brain and tunica scrapings of one animal was determined. Two guinea-pigs were inoculated intraperitoneally with the brain emulsion (half-a-brain per animal) and two with the tunica scraping suspension. If the indicating animal did not react typically, it was held for one month after the temperature had returned to normal and tested for immunity. The results of the experiments are collected in tables 3, 4 and 5.

Systematic work has not been carried out on the infectivity of the tunica or blood of "Robertson" or button fever infected guinea-pigs or of the tunica of "Hare" animals. However, on many occasions, we have demonstrated the infectivity of the tunica taken at the height of the scrotal reaction. In addition, the liver and spleen of rat-typhus animals have been proved capable of transmitting the disease, but, in these experiments, no attempt was made to remove the blood from the organs.

The results given in tables 3, 4 and 5 may be briefly summarized.

Rat Typhus:

Brain.—Virulent from the 2nd to the 22nd day after inoculation but not on the 1st day or on the 26th day or later.

Blood.—Virulent from the 3rd to the 21st day inclusive, but not before or after.

Tunica.—Virulent from the 2nd to the 12th day inclusive, but not before or after.

"*Hare*":

Brain.—Virulent from the 5th to the 15th day inclusive, but not before or after.

Blood.—Clear-cut results were not obtained, but it was infective on the 4th, 5th, 8th, 10th, and 13th days after infection.

TABLE 3.

Infectivity of Brain and Tunica of Rat-typhus-infected Guinea-pigs (Exp. 109).

	Brain. Days after Infection.											
	1	2	3	4	5	6	7	19	22	26	30	33
Result of direct test..............	N N	† P	P P	P P	† †	P P	P P	P P	P P	N N	N N	N N
Result of immunity test..........	P P	N	N N	N N		ND	ND	N N	N N	P P	P P	P P
Infectivity of brain...............	—	+	+	+		+	+	+	+	—	—	—

	Tunica. Days after Infection.																
	1	2	3	4	5	6	7	9	10	12	13	16	19	22	26	30	33
Result of direct test.....	?N ?N	N N	?P ?	P ?	P P	P P	P P	P P	† ?P	P P	† N	?P ?P	N †	N N	N N	N N	N N
Result of immunity test.	P P	N P	N P	ND N	ND	ND	ND	ND	N	ND	P	P	P	P P	P P	P P	P P
Infectivity of tunica.....	—	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—

(N = no reaction ; P = positive reaction ; † = died ; ND = not done ; + = infected ; — = not infected).

Duration of Infectivity of Brain = 22 days. Duration of Infectivity of Tunica = 12 days.

TABLE 4.

Infectivity of Brain and Blood of "Hare"-infected Guinea-pigs (Exp. 19).

	Brain. Days after Infection. 1	2	3	4	5	6	10	13	15	18	24	25
Result of direct test..............	N N	N N	N N	N N	N P	P ?	P P	?P †	N N	N N	N ?	N †
Result of immunity test..........	P P	P P	P P	P P	P N	N P	ND	N	N P	P P	P P	P
Infectivity of brain..............	—	—	—	—	—	—	+	+	—	—	—	—

	Blood. Days after Infection. 2	3	4	5	6	7	8	9	10	11	13	15	18	25
Result of direct test........................	? N	N ?	N ?	N N	? N	?P P	P ?P	? ?	?P ?P	?P ?P	N ?P	P ?	N N	N †
Result of immunity test................	? †	P P	N N	N N	P †	?N P	N P	P P	P N	?P P	P N	P P	?P ?P	P
Infectivity of blood........................	?	—	[illegible]	+	—	?	[illegible]	—	[illegible]	?	[illegible]	—	?	—

(N = no reaction; P = positive reaction; † = died; ND = not done; + = infected; — = not infected).

Duration of infectivity of brain = 15 days. Duration of infectivity of blood = 13 days.

TABLE 5.

Infectivity of Blood of Rat-typhus-infected Guinea-pigs (Exp. 95).

	Days after Infection.																		
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	21	22	23	24	25
Result of direct test...........	N N	P †	P N	P - P	N N	P P	P P	P P	† P	P P	P P	P P	P P	? †	N N	N N	N N	N N	N N
Result of immunity test.......	P P	N	N P	N N	P P	N N	N N	N N	N	N N	N N	† N	N N	P	P N	P P	P P	P P	P P
Infectivity of blood............	—	+	+	+	—	+	+	+	+	+	—	+	+	—	+	—	—	—	—

(= no reaction; P = positive reaction; † = died; ND = not done; + = infected; — = not infected).
Duration of infectivity of blood = 21 days.

The results obtained with the brains of rat-typhus-infected guinea-pigs do not differ greatly from those obtained by Nicolle and Laigret (1933). With a Toulon murine strain, the virus survived for 41 but not for 44 days and with a Mexican strain for 34 but not for 44 days. However, Philip and Parker (1938) were able to recover virus from the brains of infected guinea-pigs 120 days, but not 150 days, after inoculation of virulent material.

It is interesting to note that 4 examples of an inapparent infection were got in these experiments (rat typhus table 3, tunica. 2nd day; rat typhus, table 5, blood, 21st day; "Hare", table 4, brain, 15th day; "Hare", table 4, blood, 4th day). These results, and a few others that will not be discussed, support Nicolle's (1934) contention that an immunity test is essential in work of this kind.

The Minimal Infective Dose of the Brain of Rat-typhus-infected Guinea-pigs.

Technique.

The brain was thoroughly emulsified in a measured volume of saline and the emulsion treated in the following way:—

1. Dilutions made in saline and injected intraperitoneally into guinea-pigs.

2. Emulsion spun at 4,000 r.p.m. (Ecco-Superior-H) and the supernatant fluid or dilutions of this fluid injected intraperitoneally or intracerebrally into guinea-pigs.

3. The supernatant fluid of (2) centrifuged at 14,000 r.p.m. (Ecco Ultimo) and the supernatant fluid or dilutions of this fluid injected intracerebrally into guinea-pigs.

Table 6 summarizes the results.

The results given in Table 6 show that as little as 1/2000 of a brain contained sufficient virus to infect a guinea-pig, whereas the virus had been diluted below an infecting dose in 1/4000 of a brain. The supernatant fluid of brain mush, spun at 4,000 and 14,000 r.p.m., whilst greatly reduced in titre as compared with whole brain, was still infective (the equivalent of 1/200 of a brain, in terms of supernatant fluid, for the 4,000 r.p.m. material and the equivalent of 1/50 of a brain for the 14,000 r.p.m. sample).

Immunity Experiments.

1. In Vitro *Neutralization.*

Parker and Davis (1933) were able to neutralize the virus of Rocky Mountain spotted fever, contained in the serum of infected guinea-pigs, with convalescent serum from guinea-pigs and rabbits. The virus and serum were mixed, left for half-an-hour at room temperature, and injected intraperitoneally into guinea-pigs. By a similar technique, Monteiro (1934 1 & 2) was not able to neutralize Sao Paulo typhus virus with epidemic typhus convalescent serum. Zia and Wu (1936) found that the serum of a horse hyperimmunized against typhus by Dr. Zinsser passively protected guinea-pigs against typhus.

TABLE 6.

The M.I.D. of Brains of Rat-typhus-infected Guinea-pigs.

Material Injected.	Route.	Fractions of Brain Injected.	Result.
1. Saline emulsion of brain.....	i.p.	1/100 (see note)	+
		1/1000	+
		1/1600	+
		1/2000	+
		1/4000	—
2. Supernatant prepared by spinning (1) at 4,000 r.p.m. for ½ hour	i.p.	1/4 (see note)	+
		1/20	+
		1/200	+
		1/2000	—
	i.c.	1/100	—
		1/150	+
		1/300	+ — (*a*)
		1/400	—
		1/600	—
		1/800	—
		1/1600	—
		1/2400	—
3. Supernatant prepared by spinning (2) at 14,000 r.p.m. for ½ hour	i.c.	1/50 (see note)	+ — — — — (*b*)

(i.p. = intraperitoneally; i.c. = intracerebrally; + = positive reaction; — = no reaction and not immune at subsequent test; (*a*) = 2 experiments with 2 different brains; (*b*) = 5 experiments with 5 different brains.

Note.—The fractions given for (1) represent the actual fractions of one whole brain injected. For (2) and (3) the equivalents of 1/20, 1/50, etc. of a whole brain in terms of supernatant fluid are given.

Tests carried out by us with infective guinea-pig brain as virus and the sera of recovered guinea-pigs, rabbits or sheep as antibody were very unsatisfactory. On some occasions, 5·0 c.c. of serum was given intraperitoneally to guinea-pigs, followed in 24 hours by a small dose of brain. Little if any protection was afforded even with the use of homologous serum. The same type of result was got when brain mush and serum were mixed *in vitro* and then injected intraperitoneally. Further, no definite neutralization of the virus contained in the supernatant fluid of a brain emulsion (spun at 4,000 r.p.m. for half-an-hour) could be demonstrated when this was mixed with serum *in vitro* and the mixture injected intraperitoneally.

At one time, we thought that egg-membrane cultures would prove very satisfactory as sources of virus, but later work did not justify this view. The membranes were thoroughly emulsified in 0·85 per cent. salt solution and the emulsion spun at 1,500 r.p.m. for 4 minutes to deposit large particles. The supernatant fluid was then mixed with serum (equal parts for the intracerebral test, 0·3 c.c. injected; 1·0 c.c. virus and 1·0 c.c. or 5·0 c.c. serum for the

intraperitoneal test, the whole injected) and, after standing for half-an-hour at room temperature, the mixture was inoculated into guinea-pigs. Thirteen such tests were conducted with rat typhus, "Robertson", and "Hare" egg-membrane cultures and the sera of recovered guinea-pigs. The results were not consistent; in one test, for example, rat typhus serum neutralized rat typhus virus and "Hare" serum failed to do so, whilst at the following test (using the same batch of serum and the succeeding generation of egg-culture virus) partial protection was produced by both sera. This method appeared to have possibilities that are, perhaps, worth exploring, but in our hands had no advantage over the standard technique of directly testing the resistance of recovered guinea-pigs.

We realize that the sera with which we worked were probably of very low titre and that repeatable results would doubtless be obtained with the use of high value sera such as that produced in a horse by Zinsser and Castaneda (1933).

2. *Cross-immunity Experiments in Guinea-pigs.*

The method of conducting the cross-immunity tests was that used by most workers in the typhus field. Guinea-pigs, three to six weeks after recovery from one typhus strain, received, intraperitoneally, another strain (brain or egg-membrane) and were then observed for a further two or three weeks. We wish to stress the importance of using more than one or two animals and the necessity for many tests in work of this kind. The result of six tests on two animals per test is, in our opinion, of much greater significance than the result of one test on twelve animals. A virus, slightly "weaker" than usual, may fail to produce sufficiently good reactions to justify the separation of two strains immunologically, but it is unlikely that a "weak" virus will be used on three to six occasions. Further, it is essential that an adequate number of controls be inoculated at each test and that each react typically. As a rule, we tested three or four immunized and two or three normal guinea-pigs at one time. We have not included the controls in the tables because no experiment is recorded unless they reacted satisfactorily. Our interpretation of the results was as follows. If the temperature and scrotal reaction of the guinea-pig under test were the same or nearly the same as those of the controls, the animal was taken to be non-immune to the test-virus; if a slight rise in temperature (no scrotal swelling) took place and the rise was not maintained for more than 24 or 36 hours, the result was recorded as doubtful, and if no reaction of any kind occurred the guinea-pig was considered to be immune.

3. *Cross-immunity Experiments in Sheep.*

A comparatively small number of tests was carried out in sheep, but, as will be seen from the results given in Table 8, they confirmed those obtained in the guinea-pig. Virulent guinea-pig brain inoculated intravenously was the inoculum both at the time of the infection and test. A period of from three to six weeks after the return of the temperature to normal was allowed to elapse before

the immunity test was carried out. The result was judged on the temperature reaction only; by the method of test, scrotal swellings did not occur and no "doubtful" thermal rises were recorded.

TABLE 7.

Summary of Cross-immunity tests in Guinea-pigs.

Tested with.	Immune to.														
	Rat Typhus.			Hare.			Robertson.			Appleton.			Fièvre boutonneuse.		
	R.	?	NR.	R.	?	NR.	R.	?	NR.	R.	?	NR.	R.	?	NR.
Rat typhus.......	0	0	10	10	1	0	10	0	0	16	0	2	16	2	2
Hare............	5	3	12	0	0	12	0	0	10	0	0	8	0	0	12
Robertson........	3	0	13	0	1	9	0	0	10	N.D.			1	0	26
Appleton.........	6	5	12	0	1	3	N.D.			0	0	9	N.D.		
Fièvre boutonneuse	1	1	10	0	0	10	3	0	20	N.D.			1	0	22

R = reaction; ? = doubtful reaction; NR. = no reaction; N.D. = not done.

TABLE 8.

Cross-immunity tests in Sheep.

Tested with.	Immune to.					
	Hare.		Appleton.		Rat Typhus.	
	R.	NR.	R.	NR.	R.	NR.
Hare........................	N.D.		0	2	N.D.	
Appleton.....................	0	2	N.D.		N.D.	
Rat Typhus..................	N.D.		N.D.		0	2

R = reaction; NR. = no reaction; N.D. = not done.

The results summarized in tables 7 and 8 permit the immunological grouping of the diseases in the following manner:—

Group 1: Rat Typhus.

(*a*) Immunizes solidly against itself.

(*b*) Immunizes to a great extent against "Hare", "Robertson", "Appleton" and *fièvre boutonneuse.*

Group 2: "Hare", "Robertson", "Appleton" and fièvre boutonneuse.

(*a*) "Hare" and "Appleton" cross-immunize.

(*b*) "Hare", "Robertson" and *fièvre boutonneuse* cross-immunize.

(*c*) None of the four strains immunizes against rat typhus.

Summary.

1. The effect of the rickettsias of rat typhus, tick-bite fever, *fièvre boutonneuse*, and of two other tick-bite-fever-like diseases ("Hare" and "Appleton") on the guinea-pig, rat, mouse, rabbit, dog, sheep, and ox is recorded. Rat typhus could be maintained in the guinea-pig and rat but not in the sheep, dog or mouse. "Hare", alone of the other four strains, could be easily passaged in guinea-pigs. Only by the use of cultures from the chorio-allantoic membrane of the developing chick could consistently readable reactions be got in guinea-pigs with the tick-bite fever and *fièvre boutonneuse* strains.

2. A Weil-Felix reaction was obtained with the sera of rabbits inoculated with rat typhus, "Hare" and tick-bite fever. With rat typhus, OX19 was agglutinated by the sera of ten infected animals, OX2 by none and OXK by one serum only; with "Hare" one of nine sera agglutinated OX2, doubtfully OX19 and not OXK; one other serum agglutinated OX19 only. The serum of one of seven tick-bite-fever rabbits agglutinated, in a doubtful fashion, OX19 only. The serum of rats, infected with rat typhus, agglutinated OX19 only and that of guinea-pigs, infected with rat typhus and carrying a banal proteus in their intestine did not agglutinate any one of the three proteus OX strains.

3. Details are given of the duration of infectivity of the brain, blood, and tunica vaginalis of guinea-pigs infected with rat typhus and "Hare". The brain of rat-typhus-infected guinea-pigs was virulent after 22 days, the blood after 21 days, and the tunica after 12 days. The brain of "Hare"-infected guinea-pigs was virulent after 15 days and the blood after 13 days.

4. One two-thousandth but not 1/4000 of the brain of a rat-typhus-infected guinea-pig was virulent. Much of the virus could be deposited from brain by centrifugation at 4,000 r.p.m. for half-an-hour, and all but a trace was removed at 14,000 r.p.m. for half-an-hour.

5. Attempts to neutralize the various rickettsias *in vitro* gave unsatisfactory results.

6. Cross-immunity experiments in guinea-pigs permitted the grouping of the 5 typhus strains in the following manner:—

(*a*) *Rat-typhus.*—Immunized against itself and to a great extent against the other four typhus-like diseases.

(*b*) "*Hare*", *tick-bite fever*, "*Appleton*" and *fièvre boutonneuse.*—Gave almost complete reciprocal cross-immunity, but did not immunize against rat-typhus.

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Studies of the Rickettsias of the Typhus-Rocky-Mountain-Spotted-Fever Group in South Africa.

IV.—Discussion and Classification.

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WE are in full agreement with Pijper and Crocker (1938) when they say that " Rickettsiosis is now an important and recognized chapter of pathology ". However, a perusal of the literature indicates that a state of very considerable confusion exists and that this confusion is probably more marked when considering the South African rickettsioses; these have not received the same careful attention of the numbers of independent workers engaged on the subject in other countries.

In the preceding articles (this journal) details have been given of the experimental work carried out with a number of rickettsias isolated from several different sources. In this portion of the study, it is our intention to summarize the findings with a view to classifying the diseases and to use as a basis the criteria outlined by Pinkerton (1936).

At the outset it must be emphasised that we appreciate fully that our data are by no means complete. The work was incidental to a study of *Rickettsia ruminantium*, the cause of heartwater of cattle, sheep, and goats, and was initiated primarily to serve as a positive control for certain technique and experimental procedures which had produced negative results; but the findings are recorded as it is believed that some, at least, are of considerable importance and may be of value to other workers in the field.

1. *Cross-immunity Tests.*

It is agreed that for practical purposes the result of a reciprocal cross-immunity test is the most important single criterion for the differentiation of rickettsias. Care, however, must be exercised in the interpretation of results and it is again emphasized that an

adequate number of animals must be included in a series of tests before definite conclusions are drawn. When dealing with strains of high virulence and approximately equal infectivity for guinea-pigs, clear-cut results are usually obtained, but with strains of low infectivity considerable caution must be exercised in the final interpretation. In these cases, it is believed that the use of high-titre chorio-allantoic-membrane-cultures as the test inoculum is a decided advantage.

In table 7, part III, the results of the cross-immunity tests with the five strains investigated have been summarized. With the single exception of one guinea-pig out of 23 which reacted to *fièvre boutonneuse* and subsequently proved susceptible to the homologous virus, each strain produced a solid immunity against itself. Further, strains "Hare", "Robertson", *fièvre boutonneuse,* and "Appleton" (the latter not tested against "Robertson" and *fièvre boutonneuse*) showed practically complete reciprocal cross-immunity. These results are in striking contrast with those recorded by Pijper (1936). On the other hand, the results of the tests with the murine typhus strain were not so clear cut. Rat typhus broke down the immunity of 55 (52 completely and 3 partially) out of 59 guinea-pigs recovered from infection with any one of the other strains, i.e. only 4 out of 59 did not react at the heterologous immunity test. On the other hand, these strains produced a definite reaction in only approximately 20 per cent. of the rat-typhus-recovered guinea-pigs. These results agree partially with those recorded by Pijper and Dau (1932) and are in almost complete agreement with those recorded by Gear (1938). The significance of these results obtained with cross-immunity experiments with rat typhus is not entirely clear. One is inclined to interpret them as being due to the existence of an antigen common to the two groups. Pinkerton makes a point of issuing a warning against the too rapid acceptance of this interpretation; he obtained similar results when investigating a strain of mild spotted fever from Minnesota and he quotes Nicolle who reported that a considerable number of guinea-pigs are refractory to typhus following vaccination with *B. paratyphosus* B or the bacillus of pseudo-tuberculosis. When viewed in the light of our final conclusions that the rat typhus strain belongs to one group (the typhus group) and all the other strains belong to another group (Rocky Mountain spotted fever group), this discrepancy in the cross-immunity tests indicates the danger of using a single criterion when comparing the different rickettsias.

2. *Study of Smear Preparations of the Scrotal-sac Exudate.*

The general nature of the cellular reaction produced by each strain was practically identical with all the strains studied, but the morphology, location, and especially the distribution of the organisms showed marked differences.

In the case of murine typhus the rickettsias were longer, thinner, and more delicate with a definite tendency towards the formation of threads; they were found in large numbers only in serosa cells though single organisms were seen in neutrophiles and monocytes,

probably as a result of phagocytosis. On many occasions, large numbers of infected cells were found in preparations and there was always a tendency for the rickettsias to be aggregated in clumps; heavily infected cells contained uncountable numbers of parasites which distended the cell and displaced the nucleus. Intranuclear forms were never seen.

With all the other strains the rickettsias were shorter and plumper, more commonly diplo-bacillary or diplo-coccal and never showed any tendency to form threads. Infected cells were always rare and these included, in addition to serosa cells, monocytes, the degree of infection excluding the probability of simple phagocytosis. There was never any tendency for the organisms to be aggregated in clumps; on the contrary they were always scattered throughout the cytoplasm and the degree of infection of individual cells was never so great that the number of parasites could not be counted with comparative ease. Again, intranuclear forms were not encountered.

These observations agree with those of Gear except that he records the presence of intranuclear forms in the case of his tick-bite fever strains.

3 and 4. *Histopathology, and Location and Morphology of Organisms in Sections.*

No data are available on these two points.

5. *Study of the Organism in Tissue Culture.*

Pinkerton deals exclusively with the *in vitro* cultivation of rickettsias in serum and Tyrode in the presence of surviving cells from the tunica vaginalis of guinea-pigs. In our studies, the use of the chorio-allantoic membrane of the developing chick embryo has been explored and there appears to be no valid reason why the results obtained by the two methods are not strictly comparable.

Again, our strains divided themselves into two groups. On morphological grounds, it was exceedingly difficult to differentiate the rickettsias of these two groups; the impression was gained that large numbers of minute cocco-bacillary forms were more common in the rat-typhus cultures and that, in cultures of the other strains, the individual organisms were distinctly larger. With rat-typhus cultures individual cells were frequently distended with a dense mass of rickettsias but intranuclear forms were never found in spite of the most diligent search. The picture seen when the other strains were examined was quite different. The cytoplasm of individual cells was never distended with parasites; these were scattered in a disorderly manner, though they were present in far larger numbers than in any preparation of the scrotal-sac exudate of guinea-pigs. In addition, intranuclear forms were common. The intranuclear parasites did not differ morphologically from the intracytoplasmic, and varied in number from single individuals to dense masses which gave the distended nucleus an almost homogeneous appearance. This intranuclear habitat was a constant differentiating feature.

The rat-typhus strain, although its multiplication resulted in the death of the embryo, appeared to multiply more slowly and showed a decided tendency to die out on serial passage. This tendency was not apparent with the other strains, although a sufficiently detailed investigation was not carried out to allow of a definite opinion.

6. *Studies of Organisms in the Arthropod Vector.*

No information whatever is available on the morphology, distribution or location of any of the South African strains of rickettsia of the typhus-Rocky-Mountain-spotted-fever group in arthropod vectors.

In the case of rat typhus, apart from the negative feeding experiments of Gray (1931), no record appears in the literature of any attempt to determine experimentally whether fleas are the vectors of the typhus-like-condition harboured by rats. Apparently it has been the practice to label as murine or endemic typhus a strain of rickettsia isolated from that source. This was actually done in the case of the strain with which we worked, though fortunately we believe that our data, together with those of Gear (1938) and Gear and Becker (1938), certainly place this strain in the typhus group (Gear's and our strain originated from the same source). The fact that we have shown experimentally that the rat is susceptible to both groups of rickettsia illustrates the danger of this practice since the larva of *Amblyomma hebraeum* is well known for its extremely ubiquitous feeding habits. This tick is a known transmitter of the tick-bite fever.

In the case of tick-bite fever very little has been done experimentally even to identify the arthropod vector. Brumpt (1927) states that *Amblyomma hebraeum*, *Rhipicephalus simus*, and *Boophilus decoloratus* are transmitters of tick-bite fever but does not quote any work in support of this statement. Pijper and Dau (1934-1935) isolated a strain by the injection of an emulsion of *Rhipicephalus appendiculatus* larvae collected from an immune man. They failed, however, to transmit the disease with larvae, nymphae or adults of *Amblyomma hebraeum*. Gear and Douthwaite (1938) reported the isolation of tick-bite fever from an engorged adult dog tick, *Haemaphysalis leachi*. We isolated a rickettsia, identical with Gear's tick-bite fever strains, by feeding on a guinea-pig *Amblyomma hebraeum* nymphae collected from a hare. As far as we are aware this represents the whole of the published experimental work on the transmission of tick-bite fever by ticks.

The Weil-Felix Reaction.—According to Pijper and Crocker (1938), the titres of the sera of patients convalescent from South African epidemic typhus, South African sporadic typhus, and tick-bite fever are nearly always lower than those obtained with the sera of people who have suffered from European typhus. Pijper and Dau (1934, 1935), and Pijper and Crocker (1938) report that proteus OX2 is agglutinated almost as well as OX19, and OXK agglutination is not uncommon. In an outbreak of tick-bite fever (12 patients) recorded by Gear and Bevan (1936) low titres were obtained; the

titre for OX19 was, except in one instance, higher than that for OX2 and one serum only agglutinated OXK (1:50). Gear (1938), in a larger investigation, again shows that low titres were obtained in tick-bite fever, that OX2 and OX19 agglutinins were of equal significance and that OXK was not constantly agglutinated and never to a high titre. In an extensive series of tests of the sera of patients infected with South African epidemic (louse-borne) typhus Gear (1938) shows that OX19 is generally agglutinated to a higher titre than OX2, that high OX19 titres are not uncommon (1:3,200 - 1:25,600), and that OXK agglutination is irregular and occurs no more frequently than in febrile conditions other than typhus.

In our hands, 10 rat-typhus-infected rabbits produced agglutinins for OX19 and, although low, the titres were definite. Two rabbits produced OXK, in addition to OX19, agglutinins but in no instance did OX2 agglutinins appear.

The strain " Hare ", which had much in common with tick-bite fever, stimulated the formation of both OX2 and OX19 agglutinins in one rabbit, of OX19 agglutinins only in another, and of none at all in 7 others. The human tick-bite fever strain (Robertson) itself caused the formation of just detectable amounts of OX19 agglutinins in one of seven rabbits inoculated; the remaining six were negative.

In button fever (Durand, 1932) and in Rocky Mountain spotted fever (Maxcy, 1936) high titres are not common and OX2 and OX19 are of equal significance; however, OXK is not agglutinated. This absence of OXK agglutination in these diseases would, on Pijper's positive findings in tick-bite fever, differentiate these diseases from tick-bite fever. But there is little doubt that the Weil-Felix reaction in button fever, spotted fever and tick-bite fever is a group, as opposed to a specific, agglutination; thus one is not fully justified in stressing the OXK agglutination in tick-bite fever. If the OXK agglutination is to be used as one means of separating tick-bite fever from button fever and spotted fever, then it must also be used to group South African epidemic and endemic typhus and tick-bite fever together. Whilst, on the one hand, we have results which show that a murine strain of South African typhus probably shares an antigen with tick-bite-fever (see " cross-immunity tests in guinea-pigs "), on the other hand, we have other results which clearly separate these two diseases (see " the morphology and location of the rickettsias " and " cross-immunity tests in guinea-pigs "). Thus, until a *proteus* X strain specific for tick-bite fever is isolated, we consider that the Weil-Felix reaction should not be used as an important test in differentiating tick-bite fever from button fever and spotted fever.

8. *The Clinical Picture in the Guinea-pig.*

Rat Typhus.—In common with Dr. J. H. S. Gear, of the South African Institute for Medical Research, who gave us the strain, we have had no difficulty whatsoever in maintaining rat typhus in the guinea-pig for two-and-a-half years by brain to peritoneum

passage at 8 to 10 day intervals. In addition, we have been maintaining another murine strain, placed at our disposal by Dr. G. Blanc, of the Pasteur Institute, Casablanca, for the last six months without any difficulty. Pijper and Crocker (1938) noted that endemic typhus, in their hands, always tended to die out in the guinea-pig; however, in an earlier publication [Pijper and Dau (1935)] they make no mention of this point.

We made no observations that have not already been recorded. The incubation period, the course of the fever, the scrotal reaction and the post-mortem picture agree closely with the findings of other workers. Whilst appreciating fully that a scrotal swelling can be caused by agencies other than a typhus infection, we would mention that a temperature reaction *plus* a swelling constitutes, in our experience, a surer sign of a typhus infection than a temperature reaction alone.

Tick-bite Fever and Allied Diseases (" Robertson ", " Hare ", " Appleton " and *fièvre boutonneuse*).—The maintenance of these infections (with the possible exception of " Hare ") in the guinea-pig caused the greatest difficulty. By carrying " Robertson " (human tick-bite fever) in duplicate, we were able to hold it for about fifty generations, but at no time were we happy about it. Not infrequently, one line would die out and a fresh start would have to be made with the other line. However, there was seldom any fear of losing the strain; in addition to passaging it in guinea-pigs, we cultured it on the chorio-allantoic membrane of the chick embryo. The Appleton strain behaved in almost the same way, except that we never tried to adapt it to the egg-membrane.

With infected *fièvre boutonneuse* brain as inoculum, we never really succeeded in producing satisfactory reactions in guinea-pigs and very early in the work had to have recourse to the egg-membrane. With this, good results were nearly always obtained. " Hare " gave satisfactory results for about three years (90 passages) by the brain to peritoneum method in guinea-pigs and then died out.

Thus, a difference of importance but perhaps not of first importance, is shown in the behaviour of the viruses of rat typhus and of the *fièvre boutonneuse*—tick-bite fever group in the guinea-pig.

10. *The Clinical Picture in Other Animals.*

It is questionable whether the rat, mouse, dog or sheep are of value in differentiating murine typhus from the other four typhus-like diseases with which we worked. The rat was a suitable animal in which to maintain the murine strain but, on the other hand, both " Appleton " and " Robertson " could be passaged in this animal. On the contrary, we did not succeed in adapting " Hare " to the rat. Sufficient work was not carried out in the other animals to permit a definite statement, but no indication was got that they were of value in differentiating one disease from another.

The association of the dog with tick-bite fever is an aspect of this problem which merits discussion. Pijper insists that tick-bite fever has no association with dogs except in so far as they may act as mechanical carriers of infected ticks to the habitation of man, the ticks having picked up their infection by feeding on some unidentified reservoir in a previous developmental stage. Gear, on the other hand, emphasizes the association of his cases with the deticking of dogs, but this observation throws no light upon the susceptibility of the dogs. The fact that a strain of rickettsia was isolated from the dog tick, *H. leachi*, also is of little value since the larvae of this tick are known to feed on an exceedingly wide variety of hosts. In these studies we have pointed out our failure to prove experimentally the susceptibility of the dog, but the value of this negative finding in a small number of experiments is largely discounted by our inability to be certain of the initial susceptibility of our animals. The final elucidation of this point therefore must await the completion of work on dogs, born and bred, preferably for two generations, under tick-free conditions. On the other hand, it must be borne in mind that our strain "Appleton" was isolated from the blood of a dog showing clinical symptoms of a febrile disease.

11. *Clinical Picture in Man.*

This criterion is included in this paper merely for the sake of completeness. From a perusal of the literature it is apparent that, apart from the primary sore which may be regarded as an almost pathognomonic symptom of tick-bite fever, it would be exceedingly difficult to differentiate this disease from typhus except in classical cases.

Discussion.

Our final conclusions may be open to the criticism, possibly justifiable, that we have incorrectly labelled the various strains of rickettsia as the causal organisms of murine typhus, tick-bite fever and *fièvre boutonneuse* respectively. In the absence of "type cultures" isolated from classical cases of the diseases this is a possibility, but not, in our opinion, a probability. Our strain of rat typhus was that used by Gear in his studies, the infectivity for man and the course of the disease resulting from accidental laboratory infection being described in detail by Gear and Becker (1938). Our "Hare" and "Appleton" strains proved identical with Gear's "Robertson" strain isolated from a human being suffering from what he considered to be tick-bite fever. The strain of *fièvre boutonneuse* came from the same source as that used by Gear and was isolated from ticks in a manner similar to that described by Pijper (1936). Consequently we do not feel that any unjustifiable claims for the correct identification of the respective rickettsias have been made.

The five strains of rickettsia appear to fall into two distinct groups when due consideration is given to the sum-total of those criteria which have been the subject of detailed study. In the one group falls the murine, endemic or rat-typhus strain which has

been compared with epidemic louse-borne typhus by Gear. In the other group fall strains "Robertson" (tick-bite fever of man), "Appleton" (from a dog), "Hare" (from ticks collected from a hare) and *fièvre boutonneuse* (from ticks collected from dogs at the Pasteur Institute, Tunis). It is not suggested that the name tick-bite fever should be replaced by either the French or the American term, since the name tick-bite fever has rightly received international recognition as that applied to a specific rickettsial disease in South Africa and has come into popular usage in the country. However, it is suggested that, from a biological and etiological point of view, the relationship of the various diseases should be clearly recognized or understood. Bearing in mind Pinkerton's classification of the etiological agents of the rickettsial diseases, it is proposed that the name "*Rickettsia prowazeki* var. *mooseri*" be retained for the causative agent of South African endemic typhus and that the name "*Dermacentroxenus rickettsi* var. *pijperi*" be given to the causative agent of South African tick-bite fever. The variety name "*pijperi*" is suggested in honour of Dr. A. Pijper of Pretoria who is unquestionably the pioneer in the investigation of rickettsial diseases of man in South Africa.

If this nomenclature is adopted it is apparent that certain additional modifications will become necessary; for instance, the name "*D. rickettsi* var. *conori*" should be the name given to the rickettsia causing *fièvre boutonneuse*. Further, the generic name "*Rickettsia*" cannot be allowed for *Rickettsia ruminantium*, *R. bovis*, *R. ovina* or *R. canis*, although the general names "rickettsia" and "rickettsiosis" may be retained since all are specific genera of the family *Rickettsiaceae*. These latter rickettsias differ so markedly morphologically and biologically from those of the typhus-spotted-fever group that the separation is merited, but no generic name is suggested pending the completion of adequate comparative studies.

Summary.

1. The five strains of rickettsia are discussed and compared according to the criteria suggested by Pinkerton (1936).

2. It is concluded that they fall into two groups:

(*a*) Typhus group—endemic, murine or rat typhus.

(*b*) Rocky Mountain spotted fever group—*fièvre boutonneuse*, strains "Robertson", "Appleton" and "Hare", which are similar and show only minor strain differences.

3. A proposed nomenclature is discussed.

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REFERENCES.

ALEXANDER, R. A. (1938). Studies on the neurotropic virus of horse-sickness VI. Propagation in the developing chick embyo. *Onderstepoort Jnl. of Vet. Sc. and An. Ind.*, Vol. 11, pp. 9-19.

BARYKINE, W., KOMPANESY, A., BOTCHAROWA, A, and BAVER, H. (1938). Nouvelle méthode de culture du virus du typhus exanthematique. *Off. Internat. Hyg. publ.*, Vol. 30, pp. 326-331. (Abstract *Zentralb. f. Bakt.* 1. Ref., Vol. 130. p. 212.)

BENGTSON, I. A. and DYER, R. E. (1935). Cultivation of the virus of Rocky Mountain spotted fever in the developing chick embryo. *U.S. Pub. Health. Rep.*, Vol. 50. pp. 1489-1498.

BRUMPT, E. (1927). Précis de parasitologie. Masson and Cie. 4e édition, Paris p. 872.

COMBIESCO, D. and ANGELESCO, J. (1933). Recherches expérimentales sur le typhus exanthématique chez le chien. *C.R. Soc. Biol.*, Vol. 113, pp. 497-498.

COWDRY, E. V. (1926). Cytological studies on heartwater. 1. The observation of *Rickettsia ruminantium* in the tissues of infected animals. *11th and 12th Reports, Director of Vet. Ed. and Res. Union of S.A.* pp: 161-177.

DA CUNHA, A. M. (1934). Sur la culture des Rickettsia du typhus exanthématique de Sao Paulo dans la membrane chorio-allantoïde de l'embryon de poulet. *C.R. Soc. Biol.* Vol. 117, pp. 392-394.

DURAND, P. (1932). La réaction de Weil-Felix dans la fièvre boutonneuse. *Arch. Instit. Pasteur, Tunis.*, Vol. 20, pp. 395-421.

DURAND, P. (1933). Etude expérimentale de quelques virus exanthématiques chez le chien. *Arch. Instit. Pasteur, Tunis.*, Vol. 21, pp. 484-503.

GEAR, J. H. S. and BEVAN, C. (1936). An outbreak of tick-bite fever *S.A. Med. J.*, Vol. 10, pp. 485-488.

GEAR, J. H. S. (1938). South African typhus. *S.A.J. Med. Sci.*, Vol. 3, pp. 134-160.

GEAR, J. H. S. and BECKER, L. H. (1938). A case of typhus fever contracted in the laboratory from a virus isolated from rats. *S.A. Med. J.*, Vol. 12, pp. 57-60.

GEAR, J. H. S. and DOUTHWAITE, M. (1938). The dog tick *Haemaphysalis leachi* as a vector of tick typhus. *S.A. Med. J.*, Vol 12, pp. 53-55.

GIRAUD, P. and TANNENBAUM, S. (1937[1]). Faits concernant la réaction de Weil et Felix. *C. R. Soc. Biol.*, Vol. 125, pp. 668-670.

GIRAUD, P. and TANNENBAUM, S. (1937[2]). Le comportement du cobaye vis-á-vis de la réaction de Weil et Felix peut-il éclairer la pathogénie de cette réaction. *Arch. Instit. Pasteur, Tunis,* Vol. 26, pp. 671-675.

GRAY, F. C. (1931). A note on preliminary investigations of the Port Elizabeth type of fever. *J. Med Ass. of S.A.*, Vol. 5, pp. 814-816.

HASS, G. M. and PINKERTON, H. (1936). Spotted fever II. An experimental study of fièvre boutonneuse. *J. Exp. Med.*, Vol. 64, pp. 601-623.

MAXCY, K. F. (1936). The Weil-Felix reaction of the rabbit in the diagnosis of Rocky Mountain spotted fever. *J. Infect. Dis.*, Vol. 58, pp. 288-292.

MONTEIRO, J. L. (1934[1]). Etude comparative du "typhus exanthématique de Sao Paulo" (Rickettsiose neotrophique) et du "typhus exanthématique" d'autres lieux basée sur l'épreuve de protection par le sérum de convalescents. *C. R. Soc. Biol.*, Vol. 115, pp. 1360-1362.

MONTEIRO, J. L. (1934[a]). Étude comparative entre le "typhus exanthématique de Sao Paulo" (Rickettsiose néotropique) et le "typhus exanthématique de Chili" (Rickettsiose épidémique) par l'épreuve de protection avec des serums de convalescents. *C.R. Soc. Biol.*, Vol. 116, pp. 1131-1132.

NICOLLE, CH. AND LAIGRET, J. (1933). Conservation du virus typhique dans le cerveau des rats et des cobayes inoculés avec ces virus. *Arch. Instit. Pasteur, Tunis.*, Vol. 21, pp. 357-380.

NICOLLE, CH. (1934). Sur les conditions de la recherche des infections inapparentes chez les animaux qui ne réagissent pas visiblement á l'inoculation du virus d'une fièvre exanthématique ou d'un produit dans lequel on suppose sa présence. *Arch. Instit. Pasteur, Tunis*, Vol. 23, pp. 19-31.

PARKER, R. R. AND DAVIS, G. E. (1933). Protective value of convalescent sera of Sao Paulo exanthematic typhus against virus of Rocky Mountain spotted fever. *U.S. Pub. Health Rep.*, Vol. 48, pp. 501-507.

PHILIP, C. B. AND PARKER, R. R. (1938). The persistence of the viruses of endemic (murine) typhus, Rocky Mountain spotted fever and boutonneuse fever in tissues of experimental animals. *U.S. Pub. Health Rept.*, Vol. 53, pp. 1246-1251.

PIJPER, A. AND DAU, H, (1932). Cross immunity between South African typhus and tick-bite fever. *Brit. J. Exp. Path.*, Vol. 13, pp. 33-35.

PIJPER, A. AND DAU, H. (1934). Die fleckfieberartigen Krankheiten des südlichen Afrika. *Zentralb. f. Bakt.* 1. *Orig.*, Vol. 133, pp. 7-22.

PIJPER, A. AND DAU, H. (1935). South African typhus. *J. Hygiene.* Vol. 35, pp. 116-124.

PIJPER, A. AND DAU, H. (1936). Etude expérimentale comparée de la fièvre boutonneuse et de la tick-bite fever. *Arch. Instit. Pasteur, Tunis*, Vol. 25, pp. 388-401.

PIJPER, A. AND CROCKER, C. G. (1938). Rickettsiosis of South Africa. *S.A. Med. J.*, Vol. 12, pp. 613-630.

PINKERTON, H. (1936). Criteria for the accurate classification of the rickettsial diseases (Rickettsioses) and of their aetiological agents. *Parasitology*, Vol. 28, pp. 172-189.

ZIA, S. H. (1934). The cultivation of Mexican and European typhus Rickettsiae in the chorio-allantoic membrane of the chick embryo. *Amer. J. Path.*, Vol. 10, pp. 211-218.

ZIA, S. H. AND WU, C. J. (1936). Serum treatment of experimental typhus. *Chinese Med. J. Suppl.* 1, February, pp. 270-274.

ZINSSER, H. AND CASTANEDA, M. R. (1930). Studies on typhus fever, II. Studies on the etiology of Mexican typhus fever. *J. Exper. Med.*, Vol. 52, pp. 649-659.

ZINSSER, H. AND CASTANEDA, M. R. (1933). Studies on typhus fever, XI. A report on the properties of the serum of a horse immunized with killed formalinized rickettsia. *J. Exp. Med.*, Vol. 57, pp. 391-398.

Section III.

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The Antigenic Structure of Salmonellas obtained from Domestic Animals and Birds in South Africa.

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CONTENTS.

As far as possible an attempt has been made to present the results in the form of tables. These are given on the following pages:—

INTRODUCTION.

The significant part played by *Salmonella* infection in both man and animal and the frequency with which members of this group of organisms have been associated with outbreaks of food-poisoning in man, have led to a detailed study of their antigenic components during recent years. With the advent of reliable methods of serological analysis it has become possible to recognise several new strains of *Salmonella* and to subdivide a number of older forms into distinct types in cases where groups of these were previously grouped in a haphazard fashion under one name on either clinical, zoological or cultural grounds. Where pioneer workers had to rely largely or solely on the fermentation reactions of the types for a differentiation of the groups, a description of the organism at present can be accepted only if based on reliable serological work, involving the complete antigenic analysis of the bacterium.

Schutze (1920) pointed out the futility of grouping *Salmonellas* on clinical and zoological grounds, and showed the value of serological methods of classification. By means of absorption tests he was able to divide the organisms included in the *Aertrycke* group into a number of types. Bruce White (1926, 1929 a.b.), by adopting Schutze's types as a primary basis for study, unified the *Salmonella* taxonomy by comparison of such representative strains as he could find available. He identified these various strains and introduced a system of labelling for their different antigenic components. Kauffmann (1929 a.b., 1930 a.b.c., 1931, 1934, 1935 a.b.c., 1937) continued and extended the work initiated by Bruce White, but used a different system of labelling. Lovell (1932 a) correlated the formulae presented by these two workers by giving the equivalent numbers and letters used in the two systems. In order to obviate the confusion that was bound to occur from the existence of two separate systems of antigenic labelling the *Salmonella* Sub-committee of the International Society of Microbiology (1934) adopted Kauffmann's terminology for general use.

In the study of the specific-phase—non-specific-phase variation of Andrewes (1922, 1925) the presence of two well-defined, but mutually convertible, types of organisms was recognised within the limits of a species. This phenomenon explained several of the factors concerned with the cross-agglutinations observed in a number of different types of *Salmonella*. But investigations on the antigenic structure of bacteria were actually commenced by Smith and Reagh (1903) when they studied motile and non-motile strains of the hog-cholera bacillus. They were the first to describe flagellar and somatic agglutination as two distinct processes and to show that the same organism may contain two agglutinable substances, which have the property of producing two corresponding agglutinins in animals. They found that animals inoculated with motile strains yielded a serum which agglutinated the homologous motile organisms at a dilution of over 1:10,000 but barely affected the non-motile organisms at a 1:500 dilution. Sera prepared against non-motile forms had a titre of only 100 to 500 for both motile and non-motile strains. They recognised two types of agglutination, (1) large, loose, rapidly-appearing flocculent clumps of flagellated (motile) organisms, and (2) small, compact, dense, slowly-forming (somatic) granules of non-motile organisms. They associated the agglutinins in the sera prepared with the non-motile organisms (somatic antigen) with the bodies of the bacilli and not with the flagella. On absorbing the sera made with the motile strain with non-motile bacteria, the somatic agglutinins alone were removed, the flagellar agglutinins remaining behind.

About the same time Joos (1903) described two kinds of agglutinogen and two corresponding agglutinins in *S. typhi*. He also observed two forms of clumping associated with two different agglutinogens, apparently corresponding to the flagellar and somatic agglutination of Smith and Reagh. Moreover, Joos noticed that heating at 60° to 62° C. destroyed the antigen responsible for the large loose floccules but not the flagellar agglutinins, while this temperature had no effect on the antigen forming the small granules, but destroyed the agglutinin produced by it. Soon afterwards Beyer and Reagh (1904), also working with the hog-cholera bacillus, found that the flagellar agglutinable substance was greatly damaged by heating at 70° C. for more than 20 minutes, while the somatic substance was not affected; but the heating did not destroy the agglutinogenic property of the flagellar substance. Moreover, these workers showed that heating at 70° C. destroyed the somatic but not the flagellar agglutinins.

The importance of these findings was not fully realised until Weil and Felix (1917) observed that variation in the growth characters of *Proteus* X19 was associated with very striking serological differences. The one variant, termed by them the "H" (Hauch) form, grew as a spreading film on agar and gave rise to a marked, loose floccular agglutination with its own serum; while the other variant, the "O" (ohne Hauch) form, grew as circular clumps with is own serum. They called the agglutinable substance present in the "O" form, "O" receptors and the material responsible for the large floccules of the "H" forms, "H" receptors. They showed that the "H" forms contained both receptors,

while the "O" forms contained only the "O" receptor. Sera of rabbits inoculated with the "H" variant of *Proteus X*19 contained agglutinins for both "H" and "O" forms, while rabbits injected with the "O" variant, produced agglutinins for the "O" form alone. When the "O" variant was heated at 100° C. or exposed to dilute acids or to pure alcohol its agglutinative power remained unaltered, but when the "H" form was similarly treated or grown on phenol-agar it lost its power of agglutinating in large, loose floccules but retained the property of forming small granules.

These results showed the complete analogy between the motile and non-motile forms of the hog-cholera bacillus described by Smith and Reagh and the "H" and "O" forms of Weil and Felix. Soon afterwards Braun and Schaeffer (1919) demonstrated that the "H" antigen occurs only in cultures of motile organisms, while the "O" antigen is present in both motile and non-motile cultures.

Later Weil and Felix (1920) demonstrated the presence of similar antigens in organisms of the typhoid-paratyphoid group, an observation subsequently confirmed by Gruschka (1923), Schiff (1923), Bruce White (1925) and others. Bruce White (1926) advised the use of the term "H" antigen for the labile, flocculating flagellar form, and the term "O" antigen for the stable granular form; the corresponding agglutinins he referred to as "H" and "O" agglutinins respectively. It is now conventional to attach the label "H" to the heat-labile flagellar antigens, and the label "O" to the heat-stable somatic antigens.

A further advance with flagellar and somatic agglutination was made by Orcutt (1924a) when she confirmed the work of Smith and Reagh (1903) by using motile and non-motile strains of the hog-cholera bacillus derived from a single strain, originally motile. She employed a suspension of flagella as an agglutinogen and, by using rabbits, produced a serum containing only flagellar but no somatic agglutinins. This serum agglutinated motile strains to a titre of 1:5,000, but failed to flocculate non-motile strains at 1:40, while antisera prepared with the washed bodies agglutinated both motile and non-motile strains up to 1:1,000. Orcutt (1924b) also found that heating the free flagella at 70° C. destroyed their agglutinating power without materially altering their agglutinogenic property. On the other hand neither heating at 70° C. nor at 120° C. destroyed the agglutinating and absorbing properties of the somatic antigen. The somatic agglutinins were partly destroyed at 70° C. and completely at 75° C.; but the flagellar agglutinins, although unaffected at 70° C., were partly impaired at 75° C. The work of Craigie (1931) on the distribution of the "H" and "O" antigens in the bacterial body confirmed the results obtained by Orcutt.

Weil, Felix and Mitzenmacher (1918), while working with typhoid and paratyphoid organisms, found both "H" and "O" agglutinins in the sera of patients as well as in the sera of rabbits inoculated with whole bacilli. When bacterial suspensions heated at 100° C. were inoculated into rabbits agglutinins were formed which caused small granular flocculation of the "O" forms. These observations were subsequently confirmed by Bruce White (1926).

Andrewes (1922) found that the same culture of a pure growth of a motile *Salmonella* often contained two sets of individual bacilli with entirely different " H " antigens, the one specific for the particular race, or for a few races, while the other had wide affinities for a whole group of allied races of *Salmonella*. By picking a number of single colonies from an agar plate he succeeded in separating these two variants, which he referred to as the *specific* and the *group* phases; but on sub-cultivation, especially in fluid media, he found that each of the two phases usually mutated rapidly into organisms of both types. For the purpose of examining this phenomenon Andrewes (1925) advised the use of *specific* and *group* sera, prepared by absorbing the agglutinins not required from a serum which contained both. White (1925) showed that these phases were concerned purely with changes in the flagellar antigen, the somatic antigen being the same in both phases. Scott (1926a) showed that a strain of *thompson* occurring in a quasi-group phase could be changed into a type (specific) phase. In order to suppress the excess of group antigen he grew the strain in a powerful group serum, viz. media containing 15 parts nutrient broth and 1 part of a strong group serum. After 24 hours the supernatant fluid in the tube became clear, while a thick deposit collected at the bottom. After centrifuging the culture, another tube with group-serum-broth was inoculated and a drop was plated for individual colonies. The procedure was repeated after every 6 hours, plating a drop at each time. After a few passages a pure culture with a new phase was obtained and the deposit was no longer formed in the tube. By employing Wassén's (1935) modification of Scott's method Bruner and Edwards (1939 *a* and *b*) and Edwards and Bruner (1939) were able to demonstrate additional phases in a number of organisms that were previously regarded as monophasic.

Schutze (1922), Bruce White (1925, 1926, 1929), Kauffmann (1929a, 1930a,b,c 1935a, 1935b, 1935c, 1937 etc.) and others have pointed out that the somatic as well as the flagellar antigen of *Salmonellas* may be multiple, the somatic antigen being generally regarded as the connecting link between different races of species.

Ficker (1903) and Dreyer (1909) used broth cultures extensively as agglutinating suspensions for routine diagnosis. But the agglutination obtained should be regarded as an " H "-agglutination because liquid cultures generally contain bacteria which are more motile and better supplied with flagella for " H "-agglutination than solid cultures. Moreover, Dreyer advised the use of dead cultures killed by the addition of 0·1 per cent. formalin and exposure at 37° C. for some days. Pyper (1923), on the other hand, found that bacterial suspensions containing formalin are unsuitable for purposes of routine diagnosis—he succeeded in detecting many more positive cases of typhoid fever with the complement fixation test than with a Widal test in which he was using formalised suspensions. Later Felix and Olitsky (1928) showed that for somatic agglutination the antigen must be kept free from formalin and carbolic acid as either of these inhibit somatic agglutination in the presence of " H "-antigen. Thus, by using a formalised antigen for his test,

Pyper succeeded in detecting only those cases in which the serum contained " H " agglutinins. Those cases containing " O " agglutinins, but no " H ", failed to react.

Bien and Sonntag (1917) succeeded in killing the motile forms and in destroying the flagella by heating the organisms in 30 per cent. alcohol at 37° C.: thus leaving an almost pure " O " suspension. Braun and Schaeffer (1919) found that the addition of 0·1 per cent. phenol to agar suppressed the development of flagella and, therefore, the production of the corresponding labile antigen.

At present the agglutination test is generally regarded as a very reliable aid to the recognition and classification of pathogenic bacteria. For classification both somatic and flagellar antigens must be employed, although these are not equally important in all families. In *Bacillus proteus*, for example, the flagellar antigen can be used for distinguishing large groups, while the somatic antigen is far more specialised. In Salmonellas, on the other hand, some of the somatic antigens are very widely distributed in the group, while the flagellar antigen is much more specific (Weil and Felix, 1920, Bruce White 1926).

Both Schutze (1922) and Bruce White (1925, 1926) maintain that no reliable and safe antigenic relationship can be arrived at by means of absorption tests, unless cross-absorption and cross-agglutination methods are carried out with both strains of bacteria and their sera employed in the test, i.e., unless the complete mirror test is performed. Bruce White obtained a number of so-called " Schottmuller " strains, isolated from calves and described by Christiansen (1914) as *Paratyphus-B* (Schottmuller). By means of proper absorption tests Bruce White (1926) found these organisms to be typical *S. typhi-murium*.

The absorption of agglutinins from sera was first employed by Bordet (1899), Eisenberg and Valk (1902), Castellani (1902) and Joos (1903). Subsequently this method was extensively used by several workers for the antigenic analysis of different groups of bacteria. Boycott (1906) was one of the first workers to distinguish between *Paratyphosus B* and *Bact. Aertrycke* by its use; Bainbridge (1909) and O'Brien (1910) also adopted it for the differentiation of paratyphoid organisms, while Schutze (1920, 1922) and Bruce White (1925, 1926, 1929 a and b) made extensive use of absorption tests for the classification of *Salmonellas*. For the study of the antigenic analysis of bacteria absorption tests now play a most important and indispensable part. More detailed information on the subject of agglutination will be found in a review given by Arkwright (1931).

SALMONELLA INFECTION OF CALVES.

(1) Introduction.

In Europe, especially in Holland, Denmark and Germany, *Salmonella* infection in cattle has assumed considerable proportions in certain localities, where it tends to recur year after year in an enzootic form, causing very heavy losses among young stock. The incidence of *Salmonella* infection in adults is generally regarded as

sporadic. *Bact. enteritidis* of Gaertner is the organism commonly incriminated as the cause of calf mortality, but as this labelling frequently includes a number of closely allied serological types most of the records referring to it are incomplete and unreliable. Moreover, the members of this group cause disease in man as well as in animals, but it is seldom possible to recognise the exact type of organism involved, as a reliable antigenic description of the organism is hardly ever available. When Smith and Scott (1930) studied some of the organisms isolated from cases of calf diarrhoea and labelled *Bact. enteritidis Gaertner*, they found that these belonged to the *dublin* type.

According to Jensen (1913) a form of calf diarrhoea (Kälberruhr) has been known in Europe for more than a century. Obich (1865) was probably the first to regard the disease as infectious, but it was left to Franck (1876) to prove it. The first bacteriological study, however, was made by Jensen (1891) when he investigated a serious outbreak of Kälberruhr in Denmark; but he could not completely distinguish the bacteria obtained from the normal intestinal contents of healthy calves from those of Kälberruhr. Later Thomassen (1897) described an outbreak of calf diarrhoea in Holland associated with a bacteraemia and caused by organisms which were called "pseudo-typhoid bacilli". Soon afterwards Poels (1899) studied a disease in calves which he ascribed to *pseudocolibacilli*. He distinguished *pseudocolibacilli* from ordinary virulent *B. coli* by virtue of their higher virulence for small animals, their greater motility and their inability to ferment lactose. Later Jensen (1903, 1913) described diseases in young cattle and in calves under the term "paracolibacillosis". The organisms recovered from the tissues of affected animals fermented glucose, dulcite, mannite, maltose, xylose, rhamnose and sorbite, but not lactose or saccharose. Jensen stated that cases of meat-poisoning as well as some outbreaks of paratyphoid in man could be traced to the consumption of the meat of calves suffering from this disease. He divided the organisms into three serological groups (1) those which correspond to Gaertner's bacillus and which comprise the majority of strains, (2) those which resemble *paratyphi-B* and (3) a few strains which resemble neither Gaertner nor *paratyphi-B*.

Mohler and Buckley (1902) reported a spontaneous enzootic in cattle due to a bacillus of the *enteritidis* group. They obtained the causal organism in pure culture from the internal organs of affected animals. Schmitt (1908) isolated Gaertner-like bacilli from calves affected with septicaemia, diarrhoea and pneumonia. He regarded this disease (calf paratyphoid) as probably identical with *pseudobacillosis* of Poels and *paracolibacillosis* of Jensen. Soon afterwards Luxwolda (1913), Warnecke (1914) and Douma (1916) described cases of *enteritidis Gaertner* infection in Holland, Christiansen (1915) regarded *paracoli bacilli* as identical with bacteria of the *enteritidis-paratyphus-B* group. Meyer, Traum and Roadhouse (1916) investigated an outbreak of infectious diarrhoea among a group of hand-reared calves, from 1 to 4 days old. They incriminated as the cause *Bact. enteritidis* isolated from the blood and internal organs of the affected calves.

Miessner and Kohlstock (1812), Lutje (1926) and Lehr (1927) described outbreaks of paratyphoid disease in adult cattle. Two children became infected after receiving milk from a sick cow studied by Lutje. Lehr noticed that the agglutination titre of the sera of infected animals varied from 1:100 to 1:20,000; from the faeces of some animals, the sera of which had a titre of 1:100 to 1:200, he isolated *Gaertner bacilli* and he found the milk of a cow that excreted *Gaertner bacilli* in the faeces to be infected.

Sometimes there exists a definite relationship between the disease in adult cattle and calves. Bourmer and Doetsch (1928) described several cases of Gaertner infection in both cows and calves. A number of adult animals excreted the bacilli with the faeces, and the milk of one particular cow that had to be emergency slaughtered caused infection in man. They also described an outbreak of paratyphoid involving more than 80 people who had partaken of cheese prepared from the milk of an apparently healthy cow which was discharging *Gaertner bacilli* with her faeces. Kinloch, Smith and Taylor (1926) described a widespread outbreak of acute enteritis affecting 497 persons in Aberdeen. Milk was found to be the cause of the disease and the source of infection was traced to a cow with an indurated udder which later developed septicaemia. *Gaertner bacilli* were isolated from the faeces and the vomit of a number of patients, from the infected milk and from the udder and flesh of the cow. In order to determine whether *Gaertner bacilli* are excreted with the milk Standfuss and Wilken (1933) carefully examined the milk of two cows that were discharging large numbers of *Gaertner bacilli* in the faeces. The results were entirely negative and these workers came to the conclusion that when paratyphoid bacilli occur in the milk it is due entirely to contamination.

Rimpau (1937) studied an outbreak of acute gastro-enteritis in 80 persons of an institution due to *typhi-murium (Breslau)*. The vehicle of infection was ice-cream, and it was found that the cream used originated from a herd in which there was a calf discharging *typhi-murium*.

A detailed study of the incidence of paratyphoid in calves and adult cattle was made by Pröscholdt (1931). Calves were found to be far more susceptible to infection than adults and *Gaertner bacilli* were considered to be the most important pathogen for calves, while adult cattle infected with this organism were regarded as the principal source of meat-poisoning. Pröscholdt described two outbreaks of *Gaertner* infection spreading from adult cattle to calves. Agglutination tests carried out sometimes revealed a titre of 1:100 in healthy animals, a titre of 1:200 being regarded as suspicious, and one of 1:400 as positive. Out of 465 cases tested by Pröscholdt, 404 were positive for *Gaertner* and only 61 to *typhi-murium*. Rievel (1933) kept 4 infected carriers under observation for 18 months. In some animals the agglutination titres were as high as 1:3,200. Periodically the organisms could not be detected in the faeces, and the presence of *Gaertner bacilli* could not be demonstrated in the milk at any time.

Pallaske and Lommatzsch (1933) were unable to recover *Gaertner bacilli* from the organs of more than 47 out of 79 cases which showed pathological changes of paratyphoid. By cultivating suspected material for long periods in enrichment media they were able

to detect bacteria in a large number of cases. Out of 43 outbreaks of paratyphoid in cattle, Francke, Standfuss and Wilken (1934) found 25 due to *dublin*, 11 to *typhi-murium* and a few to *rostock*. In Italy Strozzi (1934) found *S. enteritidis* var. *dublin* as the most important causal agent of calf paratyphoid. Karsten (1933) also made a comparative study of the incidence of *Gaertner* infection in adult cattle and calves. He considered that adult cattle, unlike calves, could discharge paratyphoid bacilli with the faeces for long periods, and he emphasised the danger of such dischargers to all animals that come in contact with them. Clarenburg (1933), on investigating an outbreak of paratyphoid infection, found apparently healthy calves and cows discharging Gaertner bacilli in the faeces. On slaughtering the calves three weeks later he discovered typical necrotic foci in the liver and kidneys, but failed to isolate the organisms from the intestinal contents or internal organs; but the titre of the serum at first negative was now found to be 1:200 and 1:400. With regard to meat inspection, Clarenburg (1934) showed that it is extremely difficult to obtain *Gaertner bacilli* from the muscles of some animals in which organ cultures have yielded positive results. He considered the use of enrichment media (e.g. tetrathionate broth) essential for the recovery of *Gaertner bacilli* from the muscles.

Weber (1936) regards the walls of the stomach and intestines as predilection sites for *S. enteritidis*, and therefore attaches considerable importance to the bacteriological examination of the mucosa of all suspected cases; he claims to have succeeded in detecting many carriers by placing scrapings from the intestinal mucosa in enrichment media, when the cultivation of faeces yielded negative results. He also noticed that *Gaertner bacilli* are frequently excreted intermittently in the faeces and that a negative serological test may be obtained even when an animal discharges bacilli with the faeces. Klimmeck (1936), on studying a number of herds of cattle for paratyphoid carriers, found 47 adults and 39 calves positive; of the adults 23 discharged *Gaertner* and 23 typhi-murium, while 33 calves excreted *Gaertner* and only 5 *typhi-murium*. A very small percentage of the adult carriers gave a positive agglutination reaction, while the proportion in calves was still smaller. Knoth (1936) made a differential study of 561 strains of *Gaertner bacilli* obtained from slaughter animals, using arabinose and rhamnose broth, Bitter's whey and Stern's glycerine-fuchsin broth for his identification. He included 1 strain (0·2 per cent.) in the Jena type, 12 (2·1 per cent.) in the Rostock type, 20 (3·6 per cent.) in the Ratin type and 528 (94·1 per cent.) in the Kiel type.

In East Africa Daubney (1927) investigated a very destructive form of calf paratyphoid associated with lung lesions, necrotic foci in the liver, haemorrhagic enteritis, tumor splenis and bacteraemia. He obtained organisms of the *Salmonella enteritidis* type from the blood, internal organs and faeces of affected calves. In India Shirlaw (1935) investigated a highly fatal disease in calves caused by a member of the *Salmonella enteritidis* group. Calves ranging from 4 to 120 days old were affected. A tentative diagnosis of *S. enteritidis* was made purely on biochemical grounds. Hygienic factors were regarded to play an important rôle in the genesis of the disease.

In domestic mammals calf paratyphoid is by far the most serious and most common form of *Salmonella* infection. Of the 104 calf strains studied by me, 2 were found to be *typhi-murium*, 3 *enteritidis* and ninety-nine *enteritidis* var. *dublin*—the latter described first by Bruce White (1929). The strain described by Bruce White was isolated by Biggar from a man affected with septicaemia following an operation on his kidney. Smith and Scott (1930) recognised this organism as the cause of three cases of continued fever in man studied by them. They considered that several of the cases of so-called " Gaertner septicaemia " encountered in man were in reality due to infection with the *dublin* type of organism. Some old laboratory strains obtained from outbreaks of food-poisoning, septicaemia and meningitis, and labelled *Bact. enteritidis*, were examined by them and found to be of the *dublin* type. Six strains isolated from outbreaks of calf dysentery in Denmark which were included in the paracolon group (*B. paracoli*) of Jensen (1913) were also recognised as belonging to the *dublin* type. Smith and Scott pointed out that in the majority of cases where this organism had been isolated from man, milk was incriminated as the cause; they regarded it as having a special association with bovine animals and concluded that cows' milk was the common vehicle of human infection. Bosworth and Lovell (1931) described three outbreaks of *dublin* infection in calves in Great Britain, where *Salmonella* infection is generally regarded to be very rare. A little later Smith (1934) recorded two fatal cases in children due to infection with *dublin*. The organisms were obtained from the blood, cerebro-spinal fluid, throat swabs and pus from the pleural sac of one or other of the patients.

In South Africa, Hutcheon (1893) referred to a disease of calves in the Eastern Province of the Cape that can probably be identified with " lewersiekte " of Otto Henning (1894). Hutcheon believed that the infection was spread from farm to farm by means of the faeces of infected calves.

Otto Henning (1894) described the disease under the name of " yellow liver " or " lewersiekte ". Subsequently calf diarrhoea was reported from different parts of the country. In 1920 I (Henning. 1932) investigated an outbreak near Estcourt in Natal and found lesions of necrotic foci in the liver and acute enteritis, but the etiology remained obscure until Viljoen and Martinaglia (1926, 1928) and Martinaglia (1929) incriminated *Salmonella enteritidis*, obtained from the organs of affected calves as the cause of the malady. They regarded this organism as a frequent secondary invader affecting mostly calves whose vitality had been lowered by factors such as improper feeding, bad hygiene, piroplasmosis, and anaplasmosis. Martinaglia (1929) described outbreaks of *Salmonella* infection in horses, fowls and canaries as well as in calves. He discussed the bacteriology, symptomatology, pathology and diagnosis of the disease caused by a number of different strains, and classified the organisms almost entirely on their biochemical characters, no attempt being made to give the antigenic structure of the bacteria described. As a result of the work of Andrewes, Schutze, Bruce White, Kauffman and others, reliable analytic methods of serological comparison are now available so that I have been able to devote my time largely to the study of the antigenic structure of different strains of *Salmonella*

isolated from domestic animals in this country. But, for the sake of comparison, the biochemical characters of the organisms are also given. (Table 25.)

During the last three years no less than 102 outbreaks of calf paratyphoid were recorded in South Africa and in the majority of these the losses were considerable; from these outbreaks I have obtained 102 different strains of *Salmonella*. According to information received from different parts of the country it is quite evident that outbreaks occur which are never reported. In many cases the farmer inoculates his calves with paratyphoid vaccine as soon as he suspects the disease, and the inoculation frequently protects the animals against infection. In other instances the vaccination has little or no effect in protecting calves that are exposed in grossly infected areas or in premises harbouring a particularly virulent strain of the organism. At one time it was thought that these apparent breakdowns in immunity occurred only when the vaccine was prepared from a stock strain of *Salmonella* (*dublin*), but it was subsequently found that even vaccines prepared from local strains could not produce an immunity strong enough to resist a natural infection.

The disease is always most severe in very young calves, but it may affect calves up to 4 months old. All affected calves discharge large numbers of bacilli with their faeces resulting in their wholesale dissemination. The scourge usually commences on a farm with a few cases of acute diarrhoea, and during the ensuing years the incidence of the disease may increase to an alarming extent, depending upon the conditions under which the animals are kept. In some outbreaks the infection becomes so severe that the majority of the calves reared on the place succumb to the disease. With the increase in the number of cases of paratyphoid the locality becomes more and more heavily infected resulting in the creation of a vicious circle. Farms which contain the greatest number of cattle are generally the worst infected.

The habit of kraaling calves, or of kraaling the cows while the calves are admitted during the milking, or any procedure which permits calves under conditions where they have to come in contact with infected manure, favours infection. It is not known how long the mânure in infected premises will remain infective; all the available evidence suggests that the infection persists for a matter of years. In 1934 I inoculated a young bovine with a virulent culture of *Salmonella dublin* (strain 154). After a severe reaction the animal recovered, but remained a carrier and discharged the organisms in its faeces for several months afterwards. Some of the infected faeces were collected, spread in a thin layer over a Petri dish and dried in the incubator for 48 hours; the dried manure was scraped out, bottled and placed on a shelf in the laboratory. Periodically this manure was tested for the presence of *dublin*; this was done by inoculating some manure in an enrichment medium, like tetrathionate broth, and by spreading some of the growth obtained on MacConkey's lactose bile-salt agar. After 1,069 days the last test was made and the manure was found to be as badly infected as at the first test. Whether the organisms will survive for as long a period under natural conditions in the kraal or stable manure remains to be proved, but the

fact that, under certain conditions, *dublin* bacilli can remain alive in the manure for nearly three years is an indication that they are very resistant and that dry manure from infected premises must be regarded as very dangerous. The possibility of calves obtaining the infection from the manure under natural conditions should, therefore, be emphasised. Moreover, when cows are milked in stables or kraals with the floors covered with manure, dry or moist, contamination of the milk with manure may lead to the dissemination of *dublin* through the milk; a number of European workers (see above) have shown that the milk of cows discharging paratyphoid bacilli with the faeces may be contaminated with these bacilli, and that when milk is infected, the infection is always obtained from the faeces and not from the udder.

My observations agree with those of Daubney (1927) and Viljoen and Martinaglia (1928), viz., that exposure of calves to unfavourable conditions and to diseases like piroplasmosis and anaplasmosis predispose them to infection. I have studied several outbreaks in which the calves were so badly infested with ticks that their resistance must have been lowered considerably. Sometimes there was no doubt that the mortality could be attributed either to piroplasmosis, anaplasmosis, gonderiosis, or heartwater, but in other cases the calves were infected with *dublin* as well as one or more of the tick-borne diseases. The rôle played by the latter in predisposing calves to paratyphoid infection must, therefore, be considerable. In some of the outbreaks studied by me I consider paratyphoid as a disease *per se*, but in many I regard the tick-borne disease as the primary cause of illness and the *dublin* infection as secondary. Moreover, the hygienic conditions under which the animals are kept also play a very important part in the genesis of the disease—particularly when they are frequently exposed to conditions that bring them in contact with infected manure.

Prophylactic measures for combating calf paratyphoid, therefore, should entail the systematic eradication of ticks as well as the application of rigorous hygienic measures in all premises where calves are raised.

All excreta and infected carcases should be properly disposed of, and healthy calves should be removed from the infected premises to clean surroundings. Vaccination, although a useful method of prophylaxis, cannot be relied upon solely; its value is greatest when it is used in conjunction with the application of suitable hygienic and tick eradication measures. But, as vaccination against calf paratyphoid forms the subject of another paper which is being prepared in collaboration with other workers at Onderstepoort, it will not be discussed here.

From these records it is clear that *Salmonellas* are common pathogens of calves in different parts of the world, generally setting up symptoms of septicaemia, acute diarrhoea, pneumonia, and meningitis with lesions of haemorrhagic enteritis, broncho-pneumonia, tumor splenis, necrotic foci in the liver and kidneys, and meningitis. In the vast majority of outbreaks described, *S. enteritidis* is incriminated as the cause of the disease; but, apart from the work of Bruce White (1929), Smith and Scott (1930), Bosworth and Lovell (1931),

Smith (1934), Kauffmann (1935b, 1935c) and a few others, the identification of the organism was not based on its serological characters. On the basis of a series of agglutination absorption tests carried out with all the strains of *Salmonella* obtained from calves in South Africa, I have been able to recognise the organism responsible for each outbreak. The results of these tests are given in Tables 1, 2 and 3.

(2) The Technique Employed.

The material studied was obtained from different parts of the country. In most cases it was composed of organ specimens (liver and spleen) sent to the laboratory in 50 per cent. glycerine; sometimes fresh faeces or faeces sent in glycerine were submitted for examination. Occasionally a sick animal was available for investigation. In addition several cultures made from fowls by Mr. J. D. W. A. Coles, Chief of the Department of Poultry Diseases at Onderstepoort, were studied. These are described in Section VI. Most of the material was obtained from places 100 to 800 miles away from the laboratory so that it was not possible to visit more than one or two infected farms. As routine preventive inoculation of all calves in areas infected with paratyphoid was generally carried out, it was not possible to obtain sick calves for observation that had not been previously inoculated with paratyphoid vaccine.

For the identification of *Salmonella* types the technique advised by Scott (1934) and modified by me was ususally employed. Specimens of suspected material (blood, liver, spleen, faeces) were spread directly on MacConkey's lactose bile-salt agar in Mason tubes (Mason 1933)—Scott used Petri plates. Generally it is advisable to dilute some of the material in saline or broth before it is spread on the MacConkey. In this way isolated colonies will be obtained more easily. In addition material (especially faeces) is inoculated into an enrichment medium, e.g. tetrathionate broth or 1 per cent. peptone water containing brilliant green (1 in 150,000). After 18 to 24 hours incubation the Mason tubes are examined and the enriched cultures are spread on dry MacConkey agar. The characteristic pale, finely structured *Salmonella* colonies are picked from the tube which is frequently crowded with colonies of lactose-fermenting *B. coli;* sometimes colonies of late lactose fermenting or non-lactose fermenting *B. coli*, *B. pyocyaneus* and *B. proteus* are seen—these should be avoided and should not be confused with *Salmonellas*. The suspected *Salmonella* colonies are now subjected to an agglutination test. A portion of a suspected colony is picked and emulsified in a loopful of group serum (e.g. European *cholerae-suis* serum) and in a loopful of type serum (e.g. *enteritidis* serum) on a glass slide, the dilution of the serum depending on the titre—about 1 in 50 if the titre is 1:5,000. A number of the suspected colonies are emulsified each in two separate loopfuls of diluted sera (group and type); the amount of serum carried over from the one to the other drop is too small to confuse the reaction.

Some of the colonies may agglutinate with one or other of the two drops of serum; while others may fail to agglutinate with either, or may exhibit a mere trace of agglutination. Organisms which

occur in the specific phase will react with their own type sera, while those that happen to be in the non-specific phase will agglutinate with a group serum. When a reaction occurs a characteristic flocculation is seen which is readily distinguished from non-specific salt agglutination of Rough variants. Moreover, in a positive test flocculation will occur only in the one drop and not in the other, whereas in the case of salt agglutination clumping will be observed in both. A good hand lens and a dissecting microscope are very useful during the fishing for colonies as well as for the study of the reaction. Colonies that have given a positive reaction are picked, subcultured and studied further.

"Pure" type-specific sera can be prepared by inoculating rabbits with 6 to 8-hours old broth cultures of the organism in the specific phase. But as these sera always contain a certain amount of group agglutinin, preliminary absorption of the latter with another *Salmonella* containing the same group phase, but another type phase, is recommended. If *typhi-murium* serum, for example is absorbed with a mixture of *paratyphi-B* and *cholera-suis*, the group agglutinins will be removed leaving a "pure" type serum-dilution. If the organisms used for the absorption contain the same somatic antigen (e.g. *paratyphi-B and typhi-murium*) the "O" agglutinins will also be removed; thus preventing them from interfering with the reaction. The "pure" type serum will contain only type agglutinins, but neither "O" nor group agglutinins. For routine diagnosis a set of representative type-specific sera should be available, e.g. *paratyphi-B*, *typhi-murium*, *cholera-suis*, *newport*, *thompson*, *potsdam*, *bovis-morbificans*, *typhi*, *enteritidis* and *L2* sera. If a suspected colony gives a characteristic reaction with only one of these sera, a preliminary diagnosis is made and the culture obtained from it is studied further by means of agglutination absorption tests. If group serum is used, colonies occurring in the group phase will be detected. Occasionally more than one type *Salmonella* is present in the culture (mixed infection), but the second organism is not likely to be missed as long as a reasonable number of colonies is examined.

Sometimes, when diphasic *Salmonellas* are studied, there may be some difficulty in demonstrating the existence of specific-phase colonies, if colonies in the group phase predominate. On repeated sub-cultivation of the latter, however, an occasional colony occurring in the specific phase may be detected. But in cases like European *choleraesuis*, where the organism occurs permanently (?) in the group phase, phase dissociation will not readily take place.

For the acceleration of phase dissociation Scott (1934) recommends the use of broth containing approximately 15 per cent. group serum. Group colonies cultured in this medium yield a culture with a clear supernatant fluid and a dense deposit after 18 hours' incubation. On repeated sub-cultivation in group serum-broth, a turbid supernatant fluid may ultimately be obtained. If this turbid culture is now plated, most of the colonies resulting will be in the specific phase. Sometimes as many as 10 or 12 passages may be necessary before the phase dissociation becomes apparent.

Scott's technique was improved by Wassén (1935) and Bruner and Edwards (1939a and b).

The differentiation of monophasic organisms, like *enteritidis* and the members of its subgroups, can be carried out on similar lines. The specific serum is absorbed so that only the agglutinin factors not present in the sera of the other types are left. For example, by absorbing *enteritidis* serum with the type *moscow*, agglutinin factors *g.o.* of the Kauffmann-White Schema (1934) are removed, leaving factor *m* which is exclusively present in *enteritidis*. Several of the other members can be purified by absorption with *enteritidis* which removes factors *g.o.m.*

A pure culture of the strain studied was obtained either by picking single colonies from three successive generations of the culture on agar plates, or by single-celling the culture according to the method described by Mason (1936). Saline and thermo-agglutination tests, as well as the shape of individual colonies were studied for evidence of roughness. Unless indisputably smooth colonies could be obtained the culture was discarded. Only a few strains isolated from organ material (liver or spleen) were found to be completely rough; but several cultures obtained from faeces of infected or carrier animals turned out to be rough. All the strains studied behaved morphologically and culturally like typical *Salmonellas*.

Preparation of antigens.—For the preparation of agglutinating suspensions the technique employed is that described by Lovell (1932). For "O" suspensions a smooth strain of the organism is grown on agar contained in Mason tubes (Mason, 1933) for 24 hours at 37° C., the inoculum used being obtained from agar slope cultures. The growth is washed off with 95 per cent. alcohol and heated in a waterbath at 56° C. for 2 hours. After the suspension has been centrifuged and the alcohol poured off, the deposit is re-suspended in about one cubic centimeter of distilled water, before it is made up in saline to the opacity required. For preserving the antigen, Bruce White advises the addition of 66 per cent. of glycerine to the thick suspension in distilled water; this mixture is diluted in saline when required for use, the density of the antigen being approximately a thousand million organisms per cubic centimeter.

"H" suspensions are prepared by growing a motile strain of the organism in broth at 22° C. for 18 hours or at 37° C. for 6 to 8 hours. Frequently the culture is merely left standing on the laboratory bench over-night, during which period a suitable density is usually obtained. In the case of diphasic members of the group it is essential to pick colonies in both the specific and non-specific phases, and to prepare broth suspensions from each one. In order to obtain well separated colonies a small amount of inoculum is spread on fairly dry agar in Mason tubes and incubated over-night. Next morning about a dozen or more suitable colonies are selected and numbered; half of each colony is picked and inoculated into broth, and then transferred to the incubator for about 5 to 6 hours, while the Mason tubes are placed in a refrigerator to prevent further

growth and dissociation of the colonies. Alternatively the colonies are picked and each one is seeded into duplicate tubes of broth. After incubation the one tube is stored in the refrigerator, while the duplicate is tested. It is seldom necessary to incubate the broth tubes for more than 5 or 6 hours; if a suitable density is obtained the cultures are killed by the addition of formalin to a concentration of 0·25 per cent. and heating at 57° C. for two hours. Two parallel rows of Dreyer tubes are now placed in a rack; to each of the tubes in the one row 0·5 c.c. type serum dilution is added, and to each of the tubes of the other row a similar amount of diluted group serum is added; this is followed by the addition of 0·5 c.c. of the suspension from each of the broth cultures to a tube of serum dilution in each row. The rack is placed in a waterbath at 55° C. The cultures that are agglutinated by the type serum occur in the type phase and have been obtained from colonies in that phase, while the suspensions that flocculate with group serum have been obtained from group phase colonies. The kind of antigen, type or group, required can now be prepared by inoculating broth with the remaining half of the colony in the Mason tube, meanwhile stored in the refrigerator. In order to reduce the lag phase in the growth of the cultures the broth tubes are placed in a water-bath at 40° C. for about 10 minutes before incubation.

Group and type phase colonies may also be recognised by testing them in droplets of group and type serum on a glass slide according to the method described by Scott (1934).

" H " suspensions are made up to a density of approximately 500 million per cubic centimeter and " O " antigens up to roughly 1,000 million per cubic centimeter.

Agglutinating sera are prepared by injecting rabbits intravenously with killed bacteria 4 or 5 times at 3 or 4 day intervals. For mixed " H " and " O " sera, the antigen used is a saline suspension of an eighteen hours old agar culture. The organisms are also killed by the addition of formalin to make a concentration of 0·25 per cent. and heating at 57° C. for two hours. For the preparation of type and group sera the organism in the required phase is grown in broth for approximately 6 hours and killed before injection. But the type sera obtained always contain a certain amount of group agglutinins which should be removed by means of an organism occurring in the group phase, or by one which has the same group but a different type phase. Group phase sera are also seldom " pure ", but purification is far more difficult on account of the presence of some similar group factors in all group antigens.

For the preparation of " O " antisera the antigen consists of a boiled saline suspension of an eighteen hours old agar culture. The first dose given is usually about 100 to 200 million bacteria suspended in 1 c.c. of saline. Subsequent doses can be gradually increased until a final dosage of approximately 500 to 1,000 million bacteria is reached. The administration of larger doses does not appear to be justified. It is seldom necessary to give more than five or six injections; too many injections are liable to produce sera of titres too high for easy absorption work.

Agglutination tests are carried out in Dreyer tubes placed in a water-bath at 55° C., the lower half of the tubes being immersed in water. The agglutination of "H" suspensions results in the formation of coarse, loosely arranged floccules within a very short time, reaching its maximum in about 2 to 4 hours. The clumping of "O" suspensions occurs more slowly and is characterised by the formation of fine granules; this is best seen after the tubes have been standing in the water-bath overnight.

Saline dilutions of the serum to be tested are generally made in a series of dilution tubes. From these the serum dilutions are transferred to Dreyer tubes in 0·5 c.c. amounts. A similar amount of antigen is added to each tube. For "H" agglutination the tubes are read after standing for about 2 hours in the water-bath and for "O" agglutination the readings are taken on the following morning.

For absorption tests the absorbing organism is grown on agar in Mason tubes for about 24 hours. The agar is poured fairly thick into the Mason tubes so as to furnish a good growth. After the agar has properly set the Mason tubes are placed flat in a cupboard for about 3 or 4 days in order to allow most of the water of condensation to evaporate; alternately the tubes are put in the incubator overnight. Unless some of the water of condensation is evaporated, the surface of the agar will be too moist, and the excessive fluid on the surface of the agar will interfere with the subsequent removal of the growth. The seed material is either a fresh agar or broth culture of the organism. If the surface of the media in the Mason tubes is still moist a loopful of inoculum from the agar slant is preferred, but if the surface is dry a couple of drops from the broth culture should be used. As a rule, however, a thicker growth is obtained if a large amount of inoculum is used. The seed material is spread by means of a blunt, slightly bent Pasteur pipette flamed before use. After 24 hours' incubation a fairly thick homogeneous growth will be obtained on the surface of the agar. By using Mason tubes instead of Petri plates for culturing the organism the risk of contamination is reduced to a minimum; whereas contamination of Petri plates kept in the incubator overnight is not uncommon.

The serum to be tested is diluted to the concentration required: when the "H" titre is about 1:5000 a serum dilution of 1:25 or 1:50 is recommended. The desired amount of serum dilution is measured into a thick centrifuge tube. By means of a Pasteur pipette, approximately 25 c.m. long, with an open loop at the capillary end, the growth in the tubes is scraped off and emulsified with the serum dilution along the inside of the centrifuge tube; but great care should be taken that all the clumps of bacteria are properly broken up, so that the organisms are well distributed throughout the liquid. The suspension is now placed on the bench for an hour or more and shaken every now and then so as to ensure thorough mixing of the bacteria and the serum. Although the absorption is usually complete after an hour of two on the bench, the suspension is preferably kept in the refrigerator overnight and centrifuged the next morning at about 2,000 revolutions per minute for one hour. The clear supernatant fluid is removed with a pipette

and tested. Sometimes, especially with high titre sera, better results are obtained if the absorption is performed in stages; i.e. part of the antigen is mixed with the serum dilution at first, while the rest is emulsified in the same fluid after it has been centrifuged an hour or two later.

(3) Serology.

In Table 1 are given the results of agglutination and absorption tests that were obtained with cultures 154, 217 and 216 on the one hand and *dublin* (Knox) and *enteritidis* M.7. on the other hand. The tests carried out with cultures 154 and 216 were complete bilateral (mirror) absorption tests, while the one performed with culture 217 was a unilateral absorption. Complete mirror absorption tests were also carried out with cultures 170, 171, 173, 175, 198, 203, 295 and 430. The results obtained were identical with those given for culture 154 (Table 1) and, with the exception of those relating to culture 430 (Table 3), are not recorded separately in this paper.

Cultures 418 and 290 reacted the same way as culture 216 but the records of their tests are not given; they are regarded as identical with culture 216 serologically. The results obtained with culture 190 are given in Table 2; according to a one-sided absorption test performed with culture 502 its antigenic structure is apparently similar to that of culture 190 (see below). Unilateral absorptions were also carried out with the other 87 calf strains against *dublin* (Knox) serum; the results obtained with these show that they are identical with culture 217 and are therefore not recorded here.

The records of Table I show that *Salmonella enteritidis* var. *dublin* (Knox) absorbed all the agglutinins (" O " and " H ") from 154 serum as well as from the homologous serum, while culture 154 exhausted both its own serum and *dublin* serum; *enteritidis* M.7., while completely removing all the agglutinins from the homologous serum, absorbed only the " O " agglutinins from 154 serum, leaving its " H " titre practically unchanged; in the same way, culture 154 exhausted only the " O " agglutinins from *enteritidis* serum without materially reducing the " H " titre of the latter. These results, therefore, demonstrated that culture 154 has the same " O " antigen as *enteritidis* and *dublin*, and an " H " antigen similar to that of *dublin*. Although culture 154 was agglutinated by *enteritidis* serum up to full titre, and although *enteritidis* was fully flocculated by 154 serum, *enteritidis* could not appreciably lower the " H " titre of 154 serum and culture 154 failed to remove the " H " agglutinins from *enteritidis* serum.

According to the *Salmonella* Sub-committee of the Nomenclature Committee of the International Society of Microbiology, *S. enteritidis* var. *dublin* is composed of the following antigenic factors:— " O ", IX; and " H " *gp* " O " factor XII has been added subsequently by Kauffmann (1935*b*). As strain 154 is identical with *dublin* its antigenic structure is made up of the same components. Mirror-absorption tests performed with strains 170, 171, 173, 175, 198, 203, 295 yielded the same results as strain 154; they are, therefore, also

TABLE 1.

Dublin (Knox) serum absorbed by *dublin* (Knox), and strains 154, 216 and 217.
154 serum absorbed by *dublin* (Knox), and strain 154 and *enteritidis* M.7.
216 serum absorbed by strain 216 and *enteritidis* M.7. and *dublin* (Knox).
Enteritidis M.7. serum absorbed by strain 216 and *enteritidis* M.7. and strain 154.

Antigen.	*Dublin* (Knox) Serum Absorbed by *Dublin* (Knox).	*Dublin* (Knox) Serum Absorbed by Strain 154.	*Dublin* (Knox) Serum Absorbed by Strain 217.	*Dublin* (Knox) Serum Absorbed by Strain 216.	*Enteritidis* M. 7 Serum Absorbed by *Enteritidis* M. 7.	*Enteritidis* M. 7 Serum Absorbed by Strain 154.	*Enteritidis* M. 7 Serum Absorbed by Strain 216.	154 Serum Absorbed by *Dublin* (Knox).	154 Serum Absorbed by *Enteritidis* M. 7.	216 Serum Absorbed by *Dublin* (Knox).	216 Serum Absorbed by *Enteritidis* M. 7.	154 Serum Absorbed by Strain 154.	216 Serum Absorbed by Strain 216.	*Dublin* (Knox) Serum Un-absorbed.	*Enteritidis* M. 7 Serum Un-absorbed.	154 Serum Un-absorbed.	216 Serum Un-absorbed.
Dublin Knox "O"	0	0	0	0	—	—	—	0		0	—	0	0	1,600	—	1,600	800
Dublin Knox "H"	0	0	0	6,400	—	—	—	0	—	0	—	0	0	12,800	—	25,600	6,400
Strain 154 "O"	0	0	—	—	0	0	—	0	0	—	—	0		1,600	800	1,600	—
Strain 154 "H"	0	0	—	—	0	0	—	0	12,800	—	—	0	—	12,800	12,800	25,600	—
Strain 216 "O"	0	—	—	0	0	—	0	—	—	0	0	—	0	1,600	800	—	800
Strain 216 "H"	0	—	—	0	0	—	0	—		3,200	0	—	0	12,800	12,800	—	6,400
Enteritidis "O" M. 7	—	—	—	—	0	0	0	—	0	—	0	0	0	—	800	1,600	800
Enteritidis "H" M. 7	—	—	—	—	0	6,400	0	—	0	—	0	0	0	—	12,800	25,600	6,400
Strain 217 "O"	0	—	0	—	—	—	—	—	—	—	—	—		1,600	—	—	
Strain 217 "H"	0	—	0	—	—	—	—	—	—	—	—	—	—	12,800	—	—	—

0 = less than 1 in 100.

identical with *dublin* and possess the same antigenic factors. The experiments carried out with strain 217 and the other 87 strains not recorded in Tables 1 and 3 also demonstrated that these strains resemble *dublin* antigenically. In Table I it was shown that strain 217 exhausted *dublin* serum as completely as this was done by *dublin* (Knox).

With regard to strain 216 the results in Table 1 show that it completely absorbed *enteritidis* M.7. serum as well as the homologous serum, whereas *enteritidis* M.7. exhausted agglutinins from both 216 serum and its own serum. On the other hand, strain 216 failed to reduce the " H " titre of *dublin* (Knox) serum but removed all its " O " agglutinins. *Enteritidis* M.7. and strain 216 must therefore, be regarded as identical, both containing the same antigenic factors viz. " O ", IX (XII), and " H " *gom*. Complete reciprocal absorption of the " O " agglutinins of *dublin* (Knox) and strain 216 sera was effected by cultures of these two organisms on account of the existence of identical " O " factors in them; but the " H " antigenic components, although cross-agglutinating to full titre with the two ser.., were not sufficiently related to reduce the " H " titres of the sera. The cross-agglutination observed between the " H " antigens of strain 216 and *dublin*, of strain 154 and *enteritidis* M.7. and the corresponding sera, took place by virtue of the presence of factor *g* in the antigenic complexes of both types. Cross-absorption removed only this factor, leaving the other components undisturbed, hence the high " H " agglutinin titre of the absorbed sera.

The antigenic structure of strain 190 was found to differ completely from that of both *dublin* and *enteritidis*, but it was readily agglutinated by *typhi-murium* " O " and type sera as well as by a group serum (e.g. *cholerae-suis* var. Kunzendorf serum). In Table 2 the records of an absorption test between strain 190 and *typhi-murium* are given. The results show that strains 190 not only removed all the type, group and " O " agglutinins from the homologous serum but also from *typhi-murium* (Glasgow) serum, while *typhi-murium* (Glasgow) exhausted all the agglutinins from its own serum as well as from 190 serum. It is evident, therefore, that the antigenic structure of strain 190 and *typhi-murium* (Glasgow) are identical. According to the Salmonellas Sub-committee of the International Society of Microbiology, the following antigenic components have been assigned to *typhi-murium*:— " O " IV, V and " H "-specific, *i*, " H "-non-specific, 1, 2, 3. According to Table 2 the same assignment should be allotted to strain 190.

A one-sided absorption test was performed with another diphasic strain of *Salmonella* (*culture* 502) also isolated from a calf that had died from paratyphoid. *Typhi-murium* (Glasgow) serum was used for the test. The result was that *culture* 502 completely removed all the " O " type and group agglutinins from *typhi-murium* serum. *Culture* 502 therefore contained the same antigenic components as *typhi-murium* (Glasgow).

The results recorded above clearly demonstrate that *Salmonella enteritidis* var. *dublin* is by far the most common cause of calf paratyphoid in South Africa. Of the 102 strains studied only two proved to be *S. typhi-murium*, three were classified as *S. enteritidis* and 97 were grouped under *S. enteritidis* var. *dublin*.

TABLE 2.

Typhi-murium (Glasgow) serum absorbed by *typhi-murium* (Glasgow) and by strain 190.

Strain 190 serum absorbed by *typhi-murium* (Glasgow) and by strain 190.

Antigen.	*Typhi-murium* Serum Absorbed by *Typhi-murium* (Glasgow).	*Typhi-murium* Serum Absorbed by Strain 190.	*Typhi-murium* Un-absorbed.	190 Serum Absorbed by *Typhi-murium* (Glasgow).	190 Serum Absorbed by Strain 190.	190 Serum Un-absorbed.
Typhi-murium "O"	0	0	1,600	0	0	1,600
Typhi-murium type	100	100	100,000	0	0	6,400
Typhi-murium group..	0	0	25,600	0	0	3,200
Strain 190 "O"..	0	0	1,600	0	0	1,600
Strain 190 type...	100	100	100,000	0	0	6,400
Strain 190 group..	0	0	25,600	0	0	3,200

0 = less than 1 : 100.

The antigenic properties of another strain, culture 430, isolated from the blood of a Native should also be recorded. The Native with a number of others had partaken of the meat of a calf which was suspected to have died from paratyphoid. Several of the Natives became violently ill and one, a woman, died from septicaemia. The blood of this woman was submitted to me for investigation and a *Salmonella*, strain 430, was isolated from it after enrichment in tetrathionate broth. Unfortunately no meat or part of the suspected carcass was available for bacteriological study (Henning 1938).

TABLE 3.

Antigen.	*Dublin* Serum Absorbed by *Dublin.*	*Dublin* Serum Absorbed by Strain 430.	*Dublin* Serum Un-absorbed.	430 Serum Absorbed by *Dublin.*	430 Serum Absorbed by Strain 430.	430 Serum Un-absorbed.
Dublin "O"......	0	0	1,600	0	0	800
Dublin "H".....	100	100	25,600	100	100	50,000
430 "O"........	0	0	1,600	0	0	800
430 "H"........	100	100	25,600	100	100	50,000

0 = less than 1 : 100.

Cultures of strain 430 were readily agglutinated by *dublin* "H" and "O" sera; cross-agglutination and cross-absorption tests were, therefore, carried out as shown in Table 3.

The results show that strain 430 absorbed all agglutinins ("O" and "H") from dublin serum as well as from the homologous serum, while *dublin* in the same way completely exhausted both 430 serum and its own serum. The presence of the small residues of unabsorbed agglutinins in both the *dublin* and 430 sera can be attributed to the high titres of the sera used for the test.

Strain 430 should therefore be regarded as another strain of *Salmonella enteritidis* var. *dublin*, containing the following antigenic components:— "O", IX (XII) and "H" *gp*.

For fermentation reactions see Table 25.

SALMONELLA INFECTION OF SHEEP.

In a recent paper (Henning 1936) I pointed out that *Salmonella* infection is not very common in sheep and that food-poisoning in man associated with mutton is comparatively rare. It is true that shortly after the Great War a very severe outbreak of food-poisoning was described in Germany by Fickinger (1919) and by Bruns and Gasters (1920). The source of the infection was traced to sheep, several of which were emergency-slaughtered in order to save the carcasses for human food. Organisms described to be of the "Paratyphosus B" type were isolated from the suspected mutton as well as the stools of the patients; but Bruce White (1929) regarded the organisms incriminated as *S. typhi-murium*. Severe outbreaks of *Salmonella* infection in sheep have also been described in America. Jordan (1925) reported an extensive epizootic of dysentery in lambs in Colorado and found the causal agent to be *S. typhi-murium*, while Newsom and Cross (1924, 1930, 1935) investigated several outbreaks of gastro-enteritis in lambs caused by the same organism; Newsom and Cross regarded the long railway journeys the lambs had to make and the long periods of fasting as predisposing factors; *typhi-murium* were obtained in pure culture from the heart blood and spleen of the affected lambs. The most common pathogenic *Salmonella* for sheep, however, is *S. abortus ovis*. This organism has been described by several workers in Europe, but it has not yet been recorded in South Africa; it was first described by Schermer and Ehrlich (1921), and later by Stephan and Geiger (1922), Bosworth and Glover (1925), Miessner and Baars (1927), Lovell (1931), Bosworth (1933) and Lesbouyries *et al.* (1933).

Although several cases of suspected paratyphoid in sheep have been reported from time to time very little is really known of the incidence of the disease in South African sheep. So far only two authentic cases of *Salmonella* infection in sheep have been studied in this country; both strains have been isolated by Dr. J. H. Mason at Onderstepoort, and handed to me for identification. The

serological characters of the one were recently described in full (Henning, 1936). As its " O " antigen was shown to differ from the somatic antigen of all previously described *Salmonellas*, it was admitted to species rank in compliance with the recommendations of the *Salmonella* Sub-committee of the Nomenclature Committee of the International Society of Microbiology (1934); in accordance with the suggestions of the Sub-committee, this organism was called *Salmonella onderstepoort*.

Serology.—The conclusions drawn regarding the specificity of *S. onderstepoort* were based on the following information condensed in Tables 4, 5A and 6.

" O " agglutination.—Cross-agglutination tests were performed with the heat-stable " O " antigens and " O " sera of the *Salmonella* types given in the Kauffmann-White schema of the Salmonella Sub-committee, as well as with the two newer types *S. aberdeen* (Smith, 1934) and *S. poonae* (Bridges and Scott, 1935). The results are recorded in Table 4; negative reactions are not given. Although *Onderstepoort* serum agglutinated *Senftenberg* " O " suspensions to nearly full titre, *Senftenberg* serum barely agglutinated *Onderstepoort* " O " antigen at a dilution of 1:100. *Onderstepoort* serum also gave a trace of flocculation with the " O " antigens of *Paratyphi-A* and *Enteritidis*. But the titre of *Onderstepoort* serum remained unaltered after absorption with either *Senftenberg*, *Paratyphi-A* or *Enteritidis*. On the other hand, *Onderstepoort* did not appreciably reduce the titre of *Senftenberg*. These results clearly show that *Onderstepoort* possesses an " O " antigen which does not correspond to that of any other *Salmonella* previously described.

" H " *agglutination.*—An *Onderstepoort* culture was plated on a Mason tube so as to obtain a number of individual colonies. These were tested against several type sera and against *cholerae-suis* (European) group serum. Some colonies were flocculated by the type sera of *Reading*, *Newport*, *Anatum*, while others were agglutinated by *cholerae-suis* group serum. These type-phase and group-phase colonies were now sown into separate tubes (or flasks) of broth and grown at room temperature for 18 hours, or at 37° C. for 5 to 6 hours; the cultures were killed by formalin (0·25 per cent.) and heat at 57° C. for 2 hours, as described above. On the other hand, *Onderstepoort* serum agglutinated to full titre broth cultures of the type phases of *newport*, *reading* and *anatum*, and broth cultures of the group phases of *cholerae-suis*, *reading*, *sendai*, *paratyphi-C*. A strong agglutination was obtained between *onderstepoort* serum and those antigens containing factors *e.h* of the Kauffman-White schema; when antigens containing only factor *e*, but not *h*, (e.g. *potsdam* and *brandenbury* were used the agglutination titre was much lower (Table 5A). *Onderstepoort* type serum (titre 1:6,400), also gave a low agglutination with the " H " antigens of *moscow* (1:400), *senftenberg* (1:400), *rostock* (1:200) and *derby* (1:200) but not with *dublin*; *moscow* and *derby* sera also gave a weak flocculation (1:100) with *onderstepoort* type suspension, but *senftenberg*, *derby* and *dublin* sera had no effect on it.

Table 4.—" O " Agglutination.

Sera.

Somatic Antigens.	*Onderstepoort.*	*Senftenberg.*	*Paratyphi—A.*	*Cholerae suis.*	*Enteritidis.*	*Onderstepoort* Absorbed by *Onderstepoort.*	*Onderstepoort* Absorbed by *Senftenberg.*	*Onderstepoort* Absorbed by *Paratyphi–A*	*Onderstepoort* Absorbed by *Cholerae suis.*	*Onderstepoort* Absorbed by *Enteritidis.*	*Senftenberg* Absorbed by *Onderstepoort.*	*Anatum.*	*London.*
Onderstepoort	6,400	100	50	0	± 50	0	6,400	6,400	6,400	6,400	0	0	0
Senftenberg	3,200	3,200	—	—	—	0	0	—	—	—	1,600	—	—
Paratyphi—A	100	—	800	—	—	0	—	0	—	—	—	—	—
Choleraesuis	10	—	—	1,600	—	0	—	—	0	—	—	—	—
Anatum	0	—	—	—	—	—	—	—	—	—	—	1,300	—
London	0	—	—	—	—	—	—	—	—	—	—	—	800
Enteritidis	± 50	—	—	—	1,600	0	—	—	—	0	—	—	—

0 = less than 1 : 50.

Table 5a.

" H " Antigens (Type).	Unabsorbed Type Sera.					Absorbed Type Sera.							
						Onderstepoort Absorbed by.			*Reading* Absorbed by.		*Newport* Absorbed by.		*Brandenburg* Absorbed by *Onderstepoort* Type.
	Onderstepoort.	*Reading.*	*Newport.*	*Brandenburg.*	*Potsdam.*	*Onderstepoort* Type.	*Reading* Type.	*Newport* Type.	*Onderstepoort* Type.	*Reading* Type.	*Onderstepoort* Type.	*Newport* Type.	
Onderstepoort	6,400	3,200	6,400	800	1,600	0	200	400	50	50	50	50	0
Reading	3,200	3,200	—	—	—	0	50	—	400	50	—	—	—
Newport	3,200	—	6,400	—	—	0	—	50	—	—	800	50	—
Brandenburg	200	—	—	6,400	—	0	—	—	—	—	—	—	6,400
Potsdam	400	—	—	—	12,800	—	—	—	—	—	—	—	—
Anatum	3,200	—	—	—	—	—	—	—	—	—	—	—	—

0 = less than 1 : 50.

On performing absorption tests (Table 5A) the specific phase of either *reading, newport or anatum* lowered the titre of *onderstepoort* type serum from 6,400 to 200, while the specific phase of *onderstepoort* was not able to exhaust the type sera of *newport* and *reading* completely. These results show that, although the type factors *e.h.* of *reading, newport* and *anatum* are fairly well represented in *onderstepoort*, complete absorption could not be effected. Whether this is due to the presence or absence of a minor extra factor, or due to the existence of a small residue of group agglutinin in the absorbed sera, remains to be seen.

The non-specific phase serum of *onderstepoort* (titre 1:25,600) agglutinated various group antigens up to different titres (Table 6). On absorbing *onderstepoort* group serum with *binns* or *newport* (group factors 1, 2, 3) the titre for *onderstepoort* antigen was reduced from 25,600 to only 12,800, and for the group phases of *L2* and *anatum* (group factors 1, 4, 6) the reduction was from 1,600 to 400. *L2* and to 3,200, removing all group agglutinations for *binns* as well as for *L2* and *anatum*. When this partly absorbed serum was further absorbed by monophasic *cholerae-suis* the titre was further reduced to 800. By absorbing unabsorbed *onderstepoort* group serum with *cholerae-suis* the titre was lowered from 25,600 to 800, and simultaneously all the group agglutinations for *binns*, *anatum* and *L2* were exhausted. The group phases of *reading* and *sendai* (factors 1, 4, 5) also lowered the titre of *onderstepoort* group serum from 25,600 to 800.

These results suggest that the reduction in the titre of *onderstepoort* serum effected by the group phases of *binns* and *newport* was caused by their group factor 1; that the reduction produced by *L2* and *anatum* can be ascribed to their group components 1 and 4; and that the almost complete absorption brought about by the non-specific phases of *cholerae-suis*, *reading* and *sendai* shold be attributed to their group factors 1, 4, 5. It is evident that *onderstepoort* contains group factors 1, 4, 5 and not 2,.3, 6. The unabsorbed agglutinins left after absorbing *cholerae-suis* serum with *onderstepoort* can be ascribed to group factor 3 contained in *cholerae-suis;* but the presence of the residue left after absorbing *reading* group serum with *onderpoort* cannot be explained, nor is it clear why *cholerae-suis*, *reading* or *sendai* failed to exhaust *onderstepoort* serum completely unless *onderstepoort* contains an extra group factor.

According to the information recorded above *Salmonella onderstepoort* possesses an " H " specific antigen which corresponds largely to the factors *e.h.* of *reading*, *newport* and *anatum*, and it contains a non-specific antigen which is very closely related to that of *reading* and *paratyphi-C* (factors 1, 4, 5). But *onderstepoort* failed to exhaust completely the specific sera of *reading*, *newport* and *anatum*, or the non-specific sera of *reading* or *cholerae-suis* (European). On the other hand, the specific phases of *reading*, *newport* or *anatum* could not absorb all the specific agglutinations from *onderstepoort* type serum, while the group phases of *reading*, *paratyphi-C* or *choleraesuis* did not remove all the group agglutinations from *onderstepoort* group serum. It is not quite clear how to explain the residue of unabsorbed agglutinins left after these absorptions; it is possible

that the specific serum contained a small amount of group agglutinin after absorption with organisms in the specific phase, or that the specific phase of *onderstepoort* possesses some factor that is lacking in the type factors *e.h.* of *reading*, *newport* and *anatum*, or that the specific factors *e.h.* contain some component that is not present in the type phase of *onderstepoort*.

The " O " antigen of *onderstepoort* exhibited characters which do not correspond to those that have been described for any other member of the *Salmonella* group of bacteria and the numeral XIV has been assigned to this new " O " factor.

Kauffmann (1937) would not accept my assignment of specific factors *e.h.* to *onderstepoort*. He agrees that factor *e* is common to *onderstepoort*, and organisms like *eastbourne*, which contain components *e.h.* and he claims to have succeeded in completely exhausting the specific agglutinins from *onderstepoort* serum by means of a strain of *eastbourne* which occurs only in the specific phase, while *onderstepoort* failed to remove all the specific agglutinins from *eastbourne*,. He does not agree, therefore, that *onderstepoort* contains factors *e.h.* and he assigns specific factor *e* . . . to *onderstepoort*.

In my previous paper (Henning, 1936) I made the following conclusions:—" A new type of *Salmonella* has been described, which it is proposed to name *Salmonella onderstepoort*. The ' H 'specific antigen corresponds largely to factors *e.h.* of *reading*, *newport* and *anatum*. Although cross-agglutination to full titre occurred, complete cross-absorption could not be affected. Apparently the specific factors *e.h.* contain some component which is lacking in the type phase of *onderstepoort*, while the specific phase of *onderstepoort* possesses some factor in addition to *e.h.*" I proposed the following antigenic components for *onderstepoort*:—

> " ' O ' antigen XIV.
>
> ' H ' antigen (specific) *e.h.* but there is probably some small portion of *e.h.* which is lacking in *onderstepoort* and apparently *onderstepoort* contains a small addition factor which is lacking in *e.h.*
>
> ' H ' antigen (non-specific) 1, 4, 5 plus an additional factor which does not occur in *S. cholerae-suis*, *S. anatum* or *Binns* ".

In view of Kauffmann's findings I repeated some of the tests which I had previously performed with *onderstepoort*. Unfortunately I did not have available a strain of *eastbourne* which occurs only in the type phase, and all the strains of *reading*, *newport* and *anatum* of my collection were definitely diphasic. Even the strains of *newport* var. *Kottbus* labelled " specific phase " and a strain of *chester* also labelled " specific " were found to contain both phases. I had to rely, therefore, on my available strains for the tests.

The results obtained are given in Table 8, and they confirm my previous findings. The specific phase of *newport* var. *Kottbus* reduced the titre of *onderstepoort* type serum from 3,200 to 200, while the type phase of *newport* lowered it to 400, and *anatum* var. *Muenster* decreased it to 300. On the other hand, the type phase

of *onderstepoort* reduced the titre of the specific agglutinins of *newport* var. *Kottbus* serum from 3,200 to 200, of *newport* from 2,000 to 400 and of *Muenster* from 12,800 to 1,600. Accordingly, the specific phase (factors e.h.) of *newport* and *newport* var. *Kottbus* could not remove a small residue of agglutinins from *onderstepoort* serum, and *onderstepoort* failed to exhaust a small residue of agglutinins from both *newport* and *newport* var. *Kottbus* sera.

TABLE 7.

Antigen.	*Typhi-murium* Serum Absorbed by *Typhi-murium.*	*Typhi-murium* Serum Absorbed by Strain 234.	*Typhi-murium* Serum Un-absorbed.	234 Serum Absorbed by *Typhi-murium.*	234 Serum Absorbed by Strain 234.	234 Serum Un-absorbed.
Typhi-murium "O"	0	0	1,600	0	0	800
Typhi-murium type	200	200	100,000	0	0	6,400
Typhi-murium group	100	100	50,000	0	0	3,200
234—"O"........	0	0	1,600	0	0	800
234—type.........	200	200	100,000	0	0	6,400
234—group.......	100	100	50,000	0	0	3,200

The specific and non-specific "H" agglutinins of *typhi-murium* could not be completely exhausted on account of the high titre of the unabsorbed serum.

0 = less than 1 : 100.

Moreover, *onderstepoort* type antigen was barely agglutinated by the serum of *abortus-equi* (factors *enx*) at 1:400, while the homologous titre was 1:6400; in the same way *onderstepoort* serum just flocculated *abortus-equi* "H" antigen at 1:200. When absorption tests were performed *abortus-equi* could not appreciably reduce the titre of *onderstepoort* serum and *onderstepoort* had no effect in lowering the titre of *abortus-equi* serum. Factor *e* of *abortus-equi* is, therefore, not well represented in *onderstepoort*.

It is evident from these results that the specific phases of *onderstepoort* and *newport*, although not entirely alike, have a great deal in common. The specific phase (factors *e.h.*) of *newport* is well represented in *onderstepoort*, and the specific phase of *onderstepoort* has a great deal in common with that of *newport*. If the component shared by *onderstepoort* and *newport* is represented by specific factor *e*, then *abortus-equi* should be expected to lower the titre of *onderstepoort serum* for the type phase of newport. According to Table 8 *abortus-equi* failed to reduce the titre of *onderstepoort* serum for the specific phases of both *onderstepoort* and *newport*.

The specific phase of *onderstepoort*, therefore, contains a factor in addition to the small one which it shares with *abortus equi*. This factor comprises most of the *eh* of *newport*, but it does not correspond to the entire *eh*.

TABLE 8.

Type Antigens.	*Onderste-poort* s.a.b. *Newport* var. *Kottbus*.	*Onderste-poort* s.a.b. *Newport*.	*Onderste-poort* s.a.b. *Anatum* var. *Muen-ster*.	*Newport* var. *Kottbus* s.a.b. *Onderste-poort*.	*Newport* s.a.b. *Onderste-poort*.	*Anatum* var. *Muen-ster* s.a.b. *Onderste-poort*.	*Onderste-poort* Serum.	*Newport* Serum.	*Newport* var. *Kottbus* Serum.	*Anatum* var. *Muen-ster* Serum.	*Abortus equi* s.a.b. *Onderste-poort*.	*Abortus equi* Serum.	*Onderste-poort* Serum a.b. *ab. equi*.
Onderstepoort.............	200	400	800	0	0	0	3,200	2,000	3,200	12,800	0	400	3,200
Newport var. *Kottbus*......	0	—	—	200	—	—	3,200	—	3,200	—	—	—	—
Newport.................	—	0	—	—	400	—	3,200	2,000	—	—	—	400	3,200
Anatum var. *Nuenster*.....	—	—	0	—	—	1,600	3,200	—	—	12,800	—	—	—
Abortus equi.............	—	—	—	—	—	—	200	200	—	—	6,400	6,400	0

s. = serum ; a.b. = absorbed by ; 0 = less than 1 in 50.

The fact that *onderstepoort* " O " serum (titre=6,400) agglutinates *senftenberg* " O " suspension nearly up to full titre (1:3,200) shows that *onderstepoort* also contains an " O " factor which is present in *senftenberg;* but, *onderstepoort* " O " serum barely agglutinates *paratyphi-A* (factors 1, 11) at 1:100 and it fails to agglutinate *anatum* and *London* (factors III, X). It can be assumed, therefore, that factors II and III which are also contained in *senftenberg*, are either entirely absent or so poorly represented in *onderstepoort* that they can be disregarded. Hence it is probable that the additional factor contained in *onderstepoort* and shared by *senftenberg* is factor XIX of Kauffmann (1937).

The following antigenic analysis can, therefore, be assigned to *onderstepoort*:—

O=XIV, (XIX).

H.specific=the greater part, but not the whole of *eh* of *newport*.

H.non-specific=1, 2, 4, 5.

The second strain of *Salmonella* (*culture* 234) obtained from sheep was also tested against various " O ", type and group sera. It was found to be diphasic and was readily agglutinated by " O " sera of group B of the the Salmonella Sub-committee, by the type serum of *typhi-murium* and the group serum of *cholerae-suis* (European). Absorption tests were, therefore, carried out between culture 234 and *typhi-murium* (Glasgow) (Table 7). Culture 234 was first plated on Mason tubes and individual colonies tested for type and group phases as described above; type and group antigens were prepared from the colonies identified.

The results of Table 7 show that *typhi-murium* removed all " O " type and group agglutinins from 234 serum, as well as from the homologous serum, while culture 234 completely exhausted both *typhi-murium* serum and its own serum. Accordingly culture 234 must be regarded as identical with *typhi-murium*, and its antigenic structure should be made up of the same components, viz. " O " antigen IV, V, " H " specific antigen *i*, " H " non-specific antigen 1, 2, 3. For fermentation reactions see Table 25.

SALMONELLA INFECTION OF PIGS.

Salmonella infection is fairly common in pigs. Although Salmon and Smith's (1885) interpretation of the significance of *S. cholerae-suis* as an etiological factor of swine fever is no longer accepted, there can be no doubt that this organism is an important pathogen for pigs and a frequent cause of food-poisoning in man. On comparing the hog-cholera bacillus with other members of the *Salmonella* group Smith and Moore (1894) found that it fermented dextrose, but no lactose or sucrose ,and that it was highly pathogenic for rabbits in very small doses. Kruse (1896) described the hog-cholera bacillus under the name of *Bacillus suipestifer* and, according to him, Selander regarded this organism as the cause of

Danish swine fever. Preisz (1898) also found *Bact. suipestifer* pathogenic for pigs and he incriminated it as the cause of " Schweineseuche " (swine-fever) and " Schweineseptikaemie " in Germany.

After Salmon's isolation of the hog-cholera bacillus from the blood and internal organs of most of the cases of swine-fever studied by him, this organism was universally accepted as the cause of this malady until de Schweinitz and Dorset (1904) pointed out that a disease indistinguishable from hog-cholera could be readily produced by injecting healthy pigs with morbid material and body fluids, that had been proved to be free from organisms. Later Dorset, Bolton and McBryde (1905) showed that, while hog-cholera could be most readily transmitted by means of inoculations of blood and serum from diseased pigs, the use of cultures of the hog-cholera bacillus only sometimes produced a disease resembling hog-cholera. Whereas pigs infected by means of morbid material from diseased ones could easily transmit swine-fever to in-contact pigs, those that were infected with culture remained practically innocuous for other pigs. They further showed that the causal agent of hog-cholera was contained in the filtrates of the blood and body fluids of sick animals, and that these filtrates, although entirely free from *Bact. cholerae-suis*, were nevertheless highly infective. They regarded the hog-cholera bacillus merely as an accessory factor in the production of disease.

Bainbridge (1908) divided the members of the paratyphoid group into four sub-groups, viz. (1) *Paratyphosus-A;* (2) *Paratyphosus-B* which was indistinguishable in its cultural characteristics from *aertrycke* and *suipestifer*, but which could be differentiated by means of absorption tests; (3) *aertrycke* and *suipestifer* which were regarded as strains of the same organism indistinguishable from one another; and (4) *enteritidis* (Gaertner) sub-group easily differentiated from the preceding by means of absorption tests. Later Bainbridge (1911) and Bainbridge and O'Brien (1912) divided paratyphoid bacteria into two groups of separate organisms; the first group they regarded as identical with *B. suipestifer*, and the second similar to *Paratyphosus-B*. These workers used agglutination and absorption tests for their identification. The source of *Paratyphosus-B* was considered to be from cases of paratyphoid fever and carriers, while *suipestifer* was apparently obtained from contaminated food and cases of food-poisoning. Savage (1912) on the other hand, like Bainbridge (1908), considered that food-poisoning bacilli of the *aertrycke* type were indistinguishable from *suipestifer*.

Dammann and Stedefeder (1910) succeeded in infecting healthy pigs either by feeding or by inoculating cultures of *B. suipestifer* and transmitted swine-fever by means of filtered material. Glässer (1909) found a type of *B. suipestifer* as the cause of disease in young pigs and called the organism *Bac. paratyphi-suis*.

Dammann and Stedefeder described *B. suipestifer* (Voldagsen) as the cause of a disease in young pigs, resembling swine-fever clinically, and known as Ferkel-typhus (suckling pig disease). Although Glässer's bacillus was frequently regarded as identical with

the Voldagsen bacillus it is now known that they differ from each other in that the former is diphasic, while the latter is monophasic, occurring only in the group phase like European *cholerae-suis*. Both of them differ from *suipestifer* culturally; but serologically *Glässer* is indistinguishable from the American variety, whereas *Voldagsen* resembles the European type.

Jordan (1917), basing his division on the study of recently isolated strains, also divided paratyphoid organisms into four groups:—

(1) *Paratyphosus-A* which fermented arabinose rapidly and dulcite slowly, xylose being left unaltered; litmus milk was turned alkaline only after some time.

(2) *Paratyphosus-B* which rapidly fermented arabinose, dulcite and xylose, and turned litmus milk alkaline in a very short period.

(3) *Suipestifer* which fermented xylose rapidly, but arabinose and dulcite slowly or not at all, i.e. not sooner than after 24 hours incubation.

(4) *Enteritidis* which was indistinguishable from the *Paratyphosus-B* group culturally but not serologically.

Jordan and Victorson (1917) used lead acetate agar for the differentiation of the types of paratyphoid bacilli. All *enteritidis* strains and most *Paratyphosus-B* strains were found to blacken this medium, while all their *suipestifer* strains and typical *Paratyphosus-A* failed to do so. According to Bruce White (1926) Schutze found that the Hirschfeld bacillus and European *suipestifer* could be distinguished from the American variety by the fact that they readily blackened lead acetate.

In the course of an investigation of swine-fever Uhlenhuth and Hübener (1909) encountered a bacterium which they called *Paratyphosus-C* bacillus. They found that culturally it was indistinguishable from *B. suipestifer*, but that it was not agglutinated by either *suipestifer* or *Gaertner* serum, while its own serum was without effect on the hog-cholera bacillus. They claimed to have isolated this bacterium from the organs of swine-fever pigs, from sausages and from human, pig and calf excreta, and regarded it as similar to the organism concerned with calf dysentery. Heimann (1912) isolated a strain of the so-called *paratyphosus-C* bacillus from cases of food-poisoning at Hildesheim, following the consumption of infected pork obtained from emergency-slaughtered pigs, but Andrewes and Neave (1921) did not regard the tests employed by Heimann as sufficiently reliable for the recognition of the organisms. Bruce White (1926), on the other hand, identified some of the Hildesheim strains as European hog-cholera bacilli.

During the Great War, and subsequently, several closely related organisms were isolated from cases of paratyphoid fever, especially in Eastern Europe. In 1915 Neukirk (1918) encountered an outbreak of disease in the Turkish army and called the causal organism *Erzindjan bacillus*. Subsequently several other workers observed a similar type of organism in different localities. Weil and Saxl (1917) isolated them from a number of Russian prisoners suffering from

paratyphoid (Walhynian strain). Weil studied another type from Albania, while Dienes and Wagner (1918), on investigating an outbreak of disease among a group of Russian prisoners, encountered several strains which were agglutinated by Voldagsen serum. They regarded their organism as identical with Weil's strain, Neukirk's *erzindjan* strain and Uhlenhuth's *paratyphosus-C* bacillus. Hirschfeld (1919) also investigated an enteric-like disease in the Serbian army and called the causal organism *Bacillus paratyphosus-C*, in flagrant disregard of the original usage of this term by Uhlenhuth and Hübener (1909). Mackie and Bowen (1919) and Macadam (1919) observed a similar type of organism in Mesopotamia, while Garrow (1920) found it in East Africa. Schutze (1920, 1921) identified an organism isolated in India in 1914 as *paratyphosus-C* and divided the *Salmonella* group of organisms into two sub-groups:—(1) *Enteritidis* (Gaertner) and (2) *Paratyphosus-B*. He further divided the latter into four serological types, viz. Schottmuller, mutton, Hirschfeld and hog-cholera bacilli. In his mutton type he included the bacillus of swine typhus or animal *paratyphosus-B* (*typhi-murium*).

By means of agglutination and absorption tests Bruce White (1926) showed that the Hirschfeld strain and Weil's Albanian strain were identical diphasic organisms, while one of the cultures described by Weil and Saxl proved to be a typical *newport* strain. The *erzindjan* strain of Neukirk was also found to be a true Hirschfeld bacillus.

Tenbroeck (1920 a and b) regarded Hirschfeld's bacillus as serologically identical with the American hog-cholera bacillus, but different culturally; whereas the former fermented dulcite and arabinose and produced hydrogen sulphide, the latter failed to do so. Unlike the hog-cholera bacillus Hirschfeld's organism did not prove to be very pathogenic for rabbits. When these animals were first injected with live Hirschfeld bacilli they were resistant to subsequent inoculations of virulent *cholerae-suis*. Tenbroeck placed far more reliance on serological tests than on biochemical reactions, and on account of its serum reactions he placed the Hirschfeld bacillus in the hog-cholera group.

Andrewes and Neave (1921) noticed that the Glässer and Voldagsen strains resembled each other culturally but not serologically; Voldagsen serum was completely exhausted of all agglutinins by Glässer, while the specific agglutinins present in Glässer serum were almost entirely unaffected by saturation with Voldagsen. Glässer and Voldagsen did not produce much hydrogen sulphide, whereas this gas was readily formed by (European) *suipestifer* and *paratyphosus-C of Hirschfeld*. Andrewes and Neave divided the hog-cholera group of organisms into two sub-groups:—

Group 1 comprising American *suipestifer*, Glässer's *typhi-suis* and Hirschfeld's *paratyphosus-C*, while group 2 was composed of European *suipestifer* and the Voldagsen strain. They showed that any member of group 1 could exhaust all agglutinins from the serum of any member of group 2, while group 2 strains could not materially reduce the titre of the sera of group 1 strains for members of group 1, although the sera were completely exhausted for the members of group 2.

The various porcine strains of *Salmonella* of the hog-cholera type and those closely related human strains to which, regardless of its original usage, the term *Bacillus paratyphosus-C* is frequently applied, form a group of organisms with very close serological affinities. Although different workers have contributed towards the study of the composition of this group it was Bruce White (1926) who finally divided the members into four well-defined types:—

1. Eastern or Hirschfeld bacillus.
2. American hog-cholera bacillus.
3. European hog-cholera bacillus.
4. Glässer-Voldagsen (Ferkeltyphus) bacillus.

The differential features of these organisms were described by Bruce White (1926) and by Nabarro, White, Dyke and Scott (1929). The specific phases of the diphasic members of the group, viz. Hirschfeld bacillus, American hog-cholera bacillus, and the Ferkeltyphus bacillus (Glässer strain) are indistinguishable; the non-specific phases of the last two are identical, while the European hog-cholera bacillus and the Voldagsen strain differ from them only in so far as they lack any trace of specific phase antigen. The Hirschfeld bacillus differs from all these by the deficiency of its non-specific phase in some of the antigenic components. They all differ from each other biochemically. The Hirschfeld bacillus ferments mannite, dulcite, arabinose, but not rhamnose; the hog-cholera bacillus (both European and American) ferments mannite and rhamnose, but not dulcite and arabinose; while the Ferkel-typhus strains (Glässer-Voldagsen) ferment only arabinose and rhamnose. All strains, excepting the American hog-cholera bacillus, produce hydrogen sulphide. Recently Bruner and Edwards (1939b) have shown that a specific phase may be obtained from monophasic European *cholerae-suis*.

Tenbroeck (1920 a and b) expressed his surprise at the comparative infrequency of paratyphoid in man caused by the hog-cholera bacillus, while Savage and Bruce White (1925) also remarked upon the rarity of *suipestifer* food-poisoning in man. They considered that the slight virulence of the organism for man and the massive doses required for setting up an infection are responsible for the low incidence of the disease. According to Krüger (1932b), it was declared by Uhlenhuth (1926), at a meeting of the German Society of Microbiology, that *suipestifer* bacilli could not be regarded as very pathogenic for man; and it was stated by Ostertag that, although thousands of swine-fever pigs were slaughtered for human consumption, mass infection of man did not occur. Kruger considered that *suipestifer* frequently lives as a saprophyte in the human body, setting up an infection only when the resistance has been lowered by conditions like appendicitis. Nevertheless, *Salmonella* infection of porcine origin has been known to cause serious disease in man. Indeed, the number of human cases of infection with *cholerae-suis* recorded during recent years cannot be treated as insignificant. Apart from the number of outbreaks of paratyphoid fever in man in Eastern Europe, due to the Hirschfeld

bacillus, several cases are reported from time to time where the hog-cholera bacillus has been incriminated as the cause of the disease. Krumwiede, Provost and Cooper (1922) recorded an outbreak of paratyphoid fever in four members of a family after eating tapioca pudding. One of the patients died and *S. cholerae-suis* was isolated from the liver. It was thought that the source of the infection was pork that contaminated the pudding. Scott (1926) described four outbreaks of infection due to the European hog-cholera bacillus, involving over a hundred persons. In all the cases the source of the infection was traced to prepared meats. A fatal case of septicaemia in man caused by "*Bacillus* (*Salmonella*) *suipestifer*" (America) and resembling typhoid fever was studied by Bauer and McLintock (1929), while two cases of human infection with the America hog-cholera bacillus were reported by Nabarro, Bruce White, Dyke and Scott (1929). Another case of American *suipestifer* causing disease in man in England was described by Boycott and McNee (1936); the organisms were obtained from blood culture, but although they were diphasic they resembled the European type culturally.

Clayton, Milne and Menton (1930) recorded an outbreak of acute gastro-enteritis in eight persons following the ingestion of pork pie. Three of the cases ended fatally; from the intestines of these patients as well as from the stools of the other five, American *suipestifer* was isolated. Another case of *cholerae-suis* infection of man was described by Branham, Motyca and Devine (1930). Kuttner and Zepp (1932, 1933) reported eleven cases of *suipestifer* infection, mostly in children. Of these ten were due to the European variety, and only one was caused by the American type; *Bact. suipestifer* was obtained by blood culture from all the patients All the cases recovered excepting one which ended fatally.

In Germany Köbe (1930) described two strains of *suipestifer* obtained from cases of meat-poisoning following the ingestion of pork. The patients showed symptoms of septicaemia with gastro-enteritis, as in typhoid fever. Krüger (1932a) reviewed several outbreaks of paratyphoid fever in which *Bact. suipestifer* was incriminated as the causal agent.

Giglioli (1930) studied a number of cases of quinine resistant fever in British Guiana and found Hirschfeld bacillus in 72 out the 77 patients examined. The organisms isolated corresponded both culturally and serologically with Hirschfeld's bacillus. More recently D'Hooghe (1932) and Mattlet (1932) described a number of fatal cases of paratyphoid fever in the Belgian Congo where they incriminated Hirschfeld's bacillus as the cause, while Tenbroeck, Li and Yii (1931) recorded five cases of infection in man caused by the same organism in Peiping (China). Materna and Januschke (1925) incriminated *cholera-suis* as the cause of purulent meningitis in a man, while Ravitch and Washington (1937) described several cases of *suipestifer* septicaemia in Negro children.

In South Africa Greenfield and Judd (1936) and Henning and Greenfield (1937) have described an outbreak of food-poisoning following the ingestion of pork infected with *S. bovis-morbificans* (Basenau).

The organism was originally described by Greenfield and Judd (1936) as a new *Salmonella—suipestifer var. Afri. Aust.* But later Henning and Greenfield (1937) showed that it is not distinguishable from *bovis-morbificans*. Basenau. Cultures of the organism were tested against various " O " and " H " sera. These were agglutinated by " O " sera of organisms containing factors VI and VIII of the Kauffmann-White schema, by group sera and by the type sera of *heidelberg and bovis-morbificans.* The organism was, therefore, regarded as diphasic.

A culture was plated so as to yield several well-separated colonies after 24 hours incubation; a number of fresh colonies were picked into broth and incubated for 5 to 6 hours at 37° C. The cultures obtained were tested against a pure group serum, e.g. *Kunzendorf* serum, and also against the type sera of *heidelberg* and *bovis-morbificans*. The colonies that occurred in the group phase agglutinated with *Kunzendorf* serum, while those that occurred in the type phase were flocculated only by *heidelberg* and *bovis-morbificans* sera. Group, type and " O " suspensions were now prepared and tested against a number of sera (Table 9). It will be noticed that *heidelberg* serum agglutinated both the group and type antigens, but not the " O " antigen, that *kunzendorf* flocculated the group antigen, but neither the type nor the " O " antigen, and that *bovis-morbificans* serum agglutinated all three antigens up to a very high titre.

After suitable antisera were prepared against *Afri. Aust.* absorption tests were performed (Table 9). On absorbing *Afri. Aust.* serum with *heidelberg* all agglutinins for the type phases of both *heidelberg* and *Afri. Aust.* were exhausted, but there was hardly any reduction of the group agglutinins (from 6,400 to 3,200), and all the " O " agglutinins remained. When this partly absorbed serum was re-absorbed by *Kunzendorf* a marked reduction of group agglutinins (from 3,200 to 400) was effected, but the " O " titre remained unaltered; Kunzendorf also reduced the group titre of unabsorbed *Afri. Aust.* serum from 6,400 to 400, but it had no ecect on the type agglutinins. On the other hand, *Afri. Aust.* removed all the type, but very little of the group agglutinins from *heidelberg* serum, and it did not reduce the " O " titre. Moreover, *Afri. Aust.* absorbed most of the group agglutinins (from 3,200 to 200) from *Kunzendorf* serum without reducing its " O " titre appreciably.

On absorbing *bovis-morbificans* serum with *Afri. Aust.* and *Afri. Aust.* serum with *bovis-morbificans* all the type, group and " O " agglutinins for both organisms were completely exhausted.

It will be observed that *Kunzendorf* did not completely exhaust the group agglutinins from *Afri. Aust.* serum and that *Afri. Aust.* failed to remove all the group agglutinins from *Kunzendorf* serum. This occurrence cannot be explained as *Afri. Aust.* and *bovis-morbificans* have the same group antigenic factors, and, according to the Kauffmann-White schema, the group antigens of *Kunzendorf* and *bovis-morbificans* are identical. There was barely any " O " agglutination between *Afri. Aust.* and *Kunzendorf*, indicating that the somatic factor VI of *Kunzendorf* is either absent or poorly represented in *Afri. Aust.*

These results clearly show that *S. suipestifer* var. *Afri. Aust.* of Greenfield and Judd has the same type antigen as *heidelberg* and *bovis-morbificans* (factor *r*) and a group antigen that corresponds largely with that of *Kunzendorf* and entirely with that of *bovis-morbificans* (factors 1, 3, 4, 5), while its somatic "O" antigen resembles that of *bovis-morbificans* (factors VI, VIII). Moreover, since *Afri. Aust.* removes all agglutinins, type, group and "O", from *bovis-morbificans* serum, and *bovis-morbificans* exhausts all agglutinins from *Afri. Aust.* serum there can be no doubt that the two organisms are identical.

TABLE 10.

Antigen.	*Typhi-murium* s.a.b. *Typhi-murium.*	*Typhi-murium* s.a.b. 192.	192 s.a.b. *Typhi-murium.*	192 s.a.b. 192.	*Typhi-murium* s. Unabsorbed.	192 s. Unabsorbed.
Typhi-murium "O"	0	0	0	0	800	1,600
Typhi-murium "H" type......	100	100	0	0	100,000	6,400
Typhi-murium "H" group.....	0	0	0	0	25,000	3,200
192—"O"...................	0	0	0	0	800	1,600
192—"H" type..............	100	100	0	0	100,000	6,400
192—"H" group.............	0	0	0	0	25,000	6,400

0 = less than 1 : 100; s. = serum; a.b. = absorbed by. The "H" type titer of *typhi-murium* was so high (1 : 100,000) that it was impossible to remove a small residue (1 : 100) of the agglutinin.

The only other record of a *Salmonella* obtained from a pig is that of Robinson and Martinaglia (1932) when they described an organism isolated from a pig at Onderstepoort. A description of the antigenic structure of this organism, strain 192, was not attempted by them, but its antigenic analysis was subsequently performed by me (Table 10). It was noticed that strain 192 was agglutinated far better by *typhi-murium* than by *cholerae-suis* serum. The agglutination obtained with the latter serum was purely floccular, while with the former the agglutination was both granular and floccular, suggesting the existence of a closer relationship between strain 192 and *typhi-murium* than between it and *cholerae-suis*. Moreover, strain 192 was found to be diphasic; its type phase colonies were agglutinated by *typhi-murium* type serum, while the group phase colonies were flocculated by both *typhi-murium* and *cholerae-suis* group sera. Accordingly agglutination and absorption tests were performed as shown in Table 10, mixed "O" and "H" type and group sera being used for the tests. The results show that *typhi-murium* removed all agglutinins ("O", "H" type and "H" group) from 192 serum as well as from the homologous serum; *culture* 192 also completely exhausted both *typhi-murium* serum and its own serum. Accordingly it was evident that *culture* 192 and

typhi-murium were composed of the same antigenic structure, and that they both contained the following antigenic factors of the Kauffmann-White schema:—" O " IV, V, " H " specific *i*, " H " non-specific 1, 2, 3.

In 1933 Dr. Robinson and myself isolated another strain of *Salmonella* (*culture* 168) from the blood of pigs suffering from a septicaemic disease in the Cape Province. This organism was readily agglutinated by *cholerae-suis* serum and was found to occur entirely in the group phase. Accordingly, absorption tests were conducted as shown in Table 11.

The results show that *culture* 168 removed all agglutinins from *cholerea-suis* (European) serum as well as from the homologous serum, and that *cholerae-suis* (European) exhausted both 168 serum and its serum. Absorption tests were also carried out with the diphasic American hog-cholera bacillus and its serum. It was found that *culture* 168, while completely removing all the " O " and group agglutinins from *cholerae-suis* serum, left the " H " specific titre almost unaltered. On the other hand *cholerae-suis* (America) completely exhausted all the agglutinins (" O " and " H " non-specific) from 168 serum. The results, therefore, showed that *culture* 168 was devoid of an " H " specific antigen and that it contained the same antigenic components as *cholerae-suis* (European), viz. the following factors of the Kauffmann-White scheme:—" O ", VI, VII. " H " non-specific 1, 3, 4, 5. " H " specific *nil*.

Six other strains, cultures 365, 380, 381, 382, 383, 384, isolated from the blood of pigs during an outbreak of swine fever in the Transvaal were also studied serologically. With strain 365 complete mirror absorption tests were carried out as in the case of *culture* 168 (Table 11) and exactly similar results were obtained. With the other five strains one-sided absorption tests were performed, using both European and American hog-cholera sera. Whereas all the agglutinins (" O " and " H " non-specific) were removed from European hog-cholera serum, the " H " specific agglutinins of the American *suipestifer* serum were unabsorbed. All six strains were found to be monophasic.

These results, therefore, clearly show that strains 365, 380, 381, 382, 383, 384 resemble *cholerae-suis* (European) antigenically, containing the same antigenic factors assigned to strain 168.

For fermentation tests see Table 25.

Murray (1934) cites several different workers who have isolated *cholerae-suis* from the faeces of a small percentage of apparently healthy pigs. He states, however, that he has been unable to demonstrate the presence of *suipestifer* in normal pigs.

SALMONELLA INFECTION OF EQUINES.

In horses infection with *S. abortus-equi* is undoubtedly the most common disease caused by the genus *Salmonella*. More than forty years ago Kilborne (1893) and Smith (1893) studied an outbreak of

TABLE 11.

Antigen.	*Cholerae-suis* (European) s.a.b. *Cholerae-suis* (European).	*Cholerae-suis* (European) s.a.b. 168.	168 s.a.b. *Cholerae-suis* (European).	168 s.a.b. 168.	168 s.a.b. *Cholerae-suis* (America).	*Cholerae suis* (America) s.a.b. 168.	*Cholerae-suis* (America) s.a.b. *Cholerae-suis* (America).	*Cholerae-suis* (European) Unabsorbed.	168 Serum Unabsorbed.	*Cholerae-suis* (America) Serum Unabsorbed.
Cholerae-suis (European) " O ".	0	0	0	0	—	—	—	800	1,600	—
Cholerae-suis (European) " H ".	0	0	0	0	—	—	—	6,400	12,800	—
168—" O "...................	0	0	0	0	0	0	0	800	1,600	800
168—" H "...................	0	0	0	0	0	0	0	6,400	12,800	6,400
Cholerae-suis (America) " O "...	—	—	—	—	0	0	0	—	1,600	800
Cholerae-suis (America) "H' type	—	—	—	—	0	3,200	0	—	0	3,200
Cholerae-suis (America) " H " group......................	—	—	—	—	0	0	0	—	12,800	6,400

0 = less than 1 : 100.
s. = serum.
a.b. = absorbed by.

abortions in mares and isolated a non-lactose fermenting organism of the hog-cholera group from the vaginal discharges of the affected animals. This organism was found to be pathogenic for rabbits and was regarded as the cause of the abortions; on cultivation it formed a membranous growth with wrinkled edges on the agar.

Subsequently several different workers investigated outbreaks of infectious absortion in mares caused ostensibly by the same bacterium studied by Kilborne and Smith. Good and Corbett (1913) studied a very serious epizootic in Kentucky due to organisms of the *enteritidis* hog-cholera group, which produced nearly 100 per cent. abortions. Intravenous inoculations of cultures of this organism caused abortions in mares within 10 days. About the same time Meyer and Boerner (1913), de Jong (1913), Dassonville and Riviere (1913), van Heelsbergen (1914) and Schofield (1914) also described epizootics of abortion in mares due to Bact. abortus-equi. Later MacFadyean and Edwards (1917) discussed the relationship of infectious abortion in mares and joint-ill in foals, while Miessner and Berge (1917) and Murray (1919) also incriminated *abortus-equi* as the etiological agent of outbreaks of abortion in mares.

Apart from causing abortions in equines this organism has been found responsible for pyaemic arthritis, joint-ill, abscessation and tendo-vaginitis. While studying the etiology of infectious arthritis in colts in America, Good and Smith (1914) isolated from the pus of the joints a bacterium which resembled the causal agent of infectious abortion in mares; but from the affected synovia of one foal they obtained *streptococci* only. In the outbreaks of pyaemic arthritis in foals investigated by Schofield (1914) Gram-negative bacteria were isolated in pure culture from the synovia of the affected joints—in a few cases only, the culture yielded a mixed growth of Gram-negative bacteria and *streptococci*. The former were regarded as closely related to the bacterium of contagious abortion in mares. In Germany Miessner and Berge (1917) ascribed the cause of a severe epizootic of abortion in a stud to a Paratyphoid organism, which was isolated from the stomach and intestines of dead foetuses; they pointed out that the majority of the foals which were born alive on the affected farm developed joint-ill, but *streptococci* were regarded as the most important etiological agent, paratyphoid organisms being obtained from only one case. In a comprehensive study of contagious abortion in mares and joint-ill in foals, MacFadyean and Edwards (1917) found *Bact. abortus-equi* as the most common cause of the two diseases. They isolated this organism from the heart-blood and internal organs of several of the aborted foetuses, and also from the joints of a number of foals affected with joint-ill. Some of the horses that were immunised with *abortus-equi* for the purpose of serum production developed arthritis. Magnusson (1919) on the other hand, considered an organism, which he called *Bact. viscosum equi*, as the most common cause of joint-ill in foals.

In South Africa Martinaglia (1929) described several cases of tendo-vaginitis in adult horses due to *abortus-equi* following horse-sickness immunisation. Out of twelve cases studied in 1922, nine yielded pure cultures of *abortus-equi*, while in the remaining three a mixed infection of this organism and a streptococcus was found.

One animal, a stallion, was affected with orchitis due to *abortus-equi.* In 1925 a similar condition appeared in mules, also after immunisation against horsesickness.

Seymour (1936) also incriminated *abortus-equi* as the cause of an outbreak of pyosepticaemia in foals, while Fujimura and Hoshi (1936) described outbreaks of contagious abortion and cases of abscessation in equines due to this organism. Moreover, they reported a case of *abortus-equi* infection in man.

Although the antigenic structure of the organism incriminated in these outbreaks is not clearly given, there seems to be very little doubt that *abortus-equi*, or a very closely related bacterium, was responsible for most of the cases. The strains isolated by Martinaglia were described as actively motile; but only one of these, *culture* 219, was kept. When this strain was finally received by me it was found to have lost all its properties of motility. After preparing antisera, agglutination and absorption tests were performed with this organism and *abortus-equi WH2*. The results of these tests showed conclusively that *culture* 219 and *abortus-equi WH2* had the same somatic antigen; culture 219 removed all the " O " agglutinins from *abortus-equi WH2* serum as well as from the homologous serum, while antigen; *culture* 219 removed all the " O " agglutinins from both sera. As *culture* 219 was non-motile its serum was devoid of " H " agglutinins and it left the " H " agglutinins of *abortus-equi WH2* serum unaltered.

Apart from *abortus-equi* infection, other types of *Salmonella* are sometimes responsible for outbreaks of disease in solipeds. Thus, Moulin and Amichau (1918), Combes (1918) and Urbain, Stocanne and Chaillot (1929) described epizootics in horses due to paratyphoid bacilli. Graham, Reynolds and Hill (1919) studied a virulent outbreak in a shipment of horses and mules due to *enteritidis.* Meissner incriminated *typhi-murium* as the cause of a disease in foals and obtained this organism as well as *abortus-equi* from mares that had aborted. Moreover, Lutje (1930) isolated both *enteritidis* and *typhi-murium* from equines affected with abortion, and he obtained *enteritidis* from foals exposed to infection with calves. Standfuss (1925) and Lehr (1928) isolated paratyphoid organisms from horses that were slaughtered in emergency. Other workers like Baumann and Gratzl (1931) and Arnberger (1931) described outbreaks of gastro-enteritis in horses due to *typhi-murium*, while Edwards (1934) investigated an epizootic of infectious colitis in 3 to 7 months old foals caused by the same organism. Cernozubov, Pilipovie and Stavel (1937) claim to have isolated five strains of *typhi-murium* and four of *paratyphi-B* from diseases in horses.

But apart from causing diseases in equines *Salmonella* infection of horses may lead to serious outbreaks of food-poisoning in countries where horse flesh is used for human food. Thus, during the Great War and the years immediately following more than 25 per cent. of all outbreaks of meat-poisoning in Germany were traced to horse meat, on the other hand, the incidence of gastro-enteritis from this source has been very low during recent years (Meyer 1934, 1936). In 1923, Meyer recorded 19 outbreaks and in 1932 only one that could be ascribed to this cause. Kuppelmayr (1924) described 47 outbreaks

of food-poisoning, involving 5,440 cases and causing 63 deaths, all traced to the consumption of infected horse meat. Elkeles (1925) recorded 61 outbreaks of meat-poisoning in Germany during 1923, involving 3,093 persons and causing 20 deaths; the majority of these cases were due to horse-meat. Glage (1916) studied an epizootic of food-poisoning due to horse-meat; 392 persons were affected and there were 2 deaths. Organisms of the *paratyphus-B* group were isolated from the suspected meat and from the stools of the patients. Infection of man following the consumption of horse-meat was also described by Muller (1921). Clarenburg (1931) described two outbreaks of food-poisoning in Holland where the cause of infection was horse-meat. Uhlenhuth (1925) isolated *typhi-murium* from patients who were suffering from acute gastro-enteritis following the consumption of horse-meat, while Kauffmann and Silberstein (1934) obtained *anatum* var. *Muenster* from a person who had developed food-poisoning after a meal containing raw horse-meat. Several other outbreaks of *Salmonella* food-poisoning in man resulting from the ingestion of horse-meat has been studied in the Reichsgesundheitsamt in Germany. Many of these have followed the consumption of meat from animals slaughtered in emergency.

Recently my colleague, Mr. R. Clark, investigated an outbreak of purulent arthritis in foals in the Orange Free State (Henning and Clark, 1938). He obtained pus from the affected joints of one foal and made cultures on agar slants; the growths obtained were submitted to me for identification. These were plated on MacConkey's bile-salt agar and yielded pure cultures of a non-lactose fermenting bacterium which looked like a *salmonella*. Several of the single colonies obtained were tested against various " O ", type and group sera. They were all agglutinated by the " O " sera of group B of the Kauffmann-White schema, and it was at first thought that the organism was probably *abortus-equi*. But, on further testing, it was found that some of the colonies were agglutinated by *typhi-murium* type serum, while others were flocculated by a pure group serum, like that of *cholerea-suis* var. *Kunzendorf*. The organism (*culture* 478) therefore was diphasic. Accordingly, antisera were prepared against it for the purposes of carrying out agglutination and absorption tests.

These tests (Table 12) show that *typhi-murium* removes all " O ", " H "-type and " H- "-group agglutinins from 478 serum, while 478, although completely exhausting the " H " agglutinins froh *typhi-murium* serum, reduced the " O " titre of the serum from 800 to approximately 200. *Culture* 478 also absorbed all the type and group agglutinins from *aberdeen* serum without altering the " O " titre, and *aberdeen* exhausted all the " H " agglutinins from 478 serum, but failed to redule its " O " agglutinin content. On the other hand, *culture* 478 removed all the agglutinins (" O ", type and group) from both *storrs* and *copenhagen* sera, while both *storrs* and *copenhagen* completely exhausted 478 serum. The results of these tests, therefore, showed that *culture* 478 is identical with *Salmonella typhi-murium* var. *storrs* (Edwards, 1935), or *S. typhi-murium* var. *copenhagen* (Kauffman, 1934), containing the following antigenic formula:—" O " IV, " H "-specific *i*, " H "-non-specific 1, 2, 3. The fermentation reactions of culture 478 are given below (Table 25).

Strains of *Salmonella typhi-murium* devoid of " O " factor V were first described by Landsteiner and Levine (1932) when they studied the *Binns* strain of Schutze. Later Kauffmann (1935a) recorded 16 variants of *typhi-murium* which contained " O " factor IV, but not factor V, and he called these variants *typhi-murium* var. *Copenhagen*. About the same time Jungherr and Wilcox (1934) obtained from pigeons a strain of *typhi-murium* which reacted atypically with maltose; an antigenic analysis of this organism made by Edwards (1935) showed that it is lacking in " O " factor V. Edwards called the variant *typhi-murium* var. *Storrs*. Hohn and Hermann (1937) also recorded an outbreak of disease in pigeons due to the IV-variant of *typhi-murium*, while Hoffmann and Edwards (1937) studied an infection in rabbits caused by the same type of organism. Moreover, several cases of infection in man due to strains of *typhi-murium* devoid of " O " factor V have been described by Zahn (1935).

Both Edwards and Kauffmann found that the IV-variants exhibited biochemical reactions that are not typical for *typhi-murium* and that strains from different localities did not always react in the same way.

Edwards (1938) points out that all the recorded outbreaks of disease due to IV-variants of *typhi-murium* have occurred in man, pigeons and rabbits; no IV-variants were found among *typhi-murium* cultures obtained from horses, sheep, guinea-pigs, rats, mice, turkeys, chickens, ducks and canaries. The strain of *typhi-murium* var. *Copenhagen* (Storrs) described by me is, therefore, the first record of this organism obtained from a horse.

SALMONELLA INFECTION OF BIRDS.

Infection of birds with different types of *Salmonella* is much more varied and widespread than in mammals, and the losses sustained through this group of organisms are probably far greater than those resulting from any other cause. Epizootics in fowls due to *S. gallinarum* are extremely common in some countries; in South Africa, fowl typhoid is without doubt the most serious infectious disease of fowls, while in Europe and America *Pullorum* disease seems to be more important. Epizootics due to *Salmonellas* other than *gallinarum* and *pullorum*, although less common, may nevertheless be responsible for serious losses in all species of domestic birds. It is with a discussion of these diseases that this part of my paper is chiefly concerned. Although relatively few outbreaks of paratyphiod in pigeons, ducks and geese have been recorded in South Africa, my discussion will not be complete unless the literature relating to disease in these birds is duly reviewed. Moreover, *Salmonella* infection, other than that due to *gallinarum* and *pullorum* occurs apparently more frequently in them than in gallinaceous birds. The extensive literature relating to fowl typhoid and *pullorum* disease is not discussed in this paper; it has been fully reviewed by a number of different workers.

Salmonella infection is most common, and also most serious, in very young birds. Adult birds usually suffer from a chronic form of the disease with lesions in the ovary, testes, joints, liver and spleen; whereas in young birds septicaemia and enteritis with changes in the internal organs are more frequently observed. The infection may be the cause of serious losses in the affected flocks and of food-poisoning in man, either through the medium of infected meat or eggs. Several different species of birds may be affected, and a number of different types of *Salmonella* have been incriminated as etiological agents.

In a recent review Schaaf (1936) mentioned *typhi-murium*, *enteritidis*, *anatum*, *cholera-suis* and *abortus-equi* as the causes of paratyphoid in birds; while Edwards (1936, 1937) has found *oranienburg* as the cause of an infection in quail and *Senftenberg* responsible for a disease in turkeys. Recently I have recorded an outbreak in chickens due to *Salmonella amersfoort* (Henning, 1937). *Typhi-murium* seems to be the most common cause, with *enteritidis* next in importance; the other organisms are only rarely found.

Natural infection usually occurs by means of foods or water contaminated with the excreta of infected animals or birds; but transmission may also take place through the medium of the egg which has obtained the infection in the ovary or oviduct, or which has been contaminated by means of infected faeces. Sometimes the embryo is dead in the shell as a result of the infection, but generally the newly hatched birds develop the disease during the first few days of life. There are several predisposing factors like bad hygiene, improper feeding and infestation by parasites which favour infection; the dirty habits of water birds, probably account for the frequency of paratyphoid in ducks and geese, as well as the number of outbreaks of food-poisoning that result from the ingestion of food-stuffs containing their eggs or meat as ingredients.

Lerche (1936) considers that 5·7 per cent. of the duck eggs sold in Germany are infected with *Salmonellas*. Frequently the shell is contaminated with infected faeces and under favourable conditions the organisms penetrate from the shell into the interior of the egg; but although the yolk is an excellent culture medium, the albumen of the fresh egg is strongly bactericidal (Lachtschenko, 1909, Rettger and Sperry, 1912, and Scott, 1930). This germicidal action, however, deteriorates when the egg becomes stale and when it is exposed to warm, moist weather for more than two weeks, the organisms may penetrate into the interior and increase in number; this increase occurs only when some yolk has diffused into the albumen. The most dangerous source of infection is food which contains duck eggs as an ingredient, and in which the organisms can readily multiply, e.g. creams, custards, puddings and " Hackfleisch " that have not been sufficiently heated during the preparation; mayonnaise is too acid for bacterial growth and is, therefore, less dangerous. The danger of eating duck eggs in the raw state is obvious, but even frying or boiling may not be sufficient to kill the organisms. Lerche (1936) considers that after 5 minutes boiling the temperature of the yolk of a duck's egg may not be much more than

40° C., while Bruns and Fromme (1934) state that after boiling an egg in the shell for 3½ minutes the temperature in the interior is only 28° C., but after 5 minutes boiling it rises to about 65° C.

(1) Pigeons.

The first record of a disease in birds caused by a *Salmonella* is given by Moore (1895) in his description of a severe epizootic in pigeons due to a bacillus of the hog-cholera group. The organism was recovered from the heart-blood and internal organs of affected birds. Salmon (1904) also described a rapidly fatal disease in pigeons, due to an organism of the " enteritidis group ". Another outbreak of pigeon paratyphoid was described by Zingle (1914), when he investigated a mortality among military birds at Strassburg. Organisms of the *Paratyphoid-B* group (*typhi-murium*?-) were obtained in pure culture from the heart-blood and organs of diseased birds, but it was not quite clear whether this infection was primary or secondary as the birds were affected simultaneously with pigeon-pox. The invasion of the body by paratyphoid organisms under certain abnormal conditions is explained by Cash and Doan (1931). They have found that latent infections with *typhi-murium* become seemingly active under adverse conditions.

Subsequently several other workers described outbreaks of *Salmonella* infection in pigeons. Thus, Reitsma (1924) studied an epizootic in Holland, as a result of which the pigeons developed an ulcerative enteritis and became very much emaciated; a pure culture of an organism, labelled " *B.paratyphus-B* " (*typhi-murium*) was obtained from the liver of the affected birds. Sahaya and Willems (1927) recorded a chronic and an acute form of the disease affecting adult and young birds respectively. The adult pigeons were usually afflicted with a severe arthritis and swelling of the joints, associated with softening and atrophy of the pectoral muscles, while young birds suffered mostly from acute enteritis. A *Salmonella*, which was not identified, was isolated from the pus of the joints in the chronic cases and from the heart-blood of the young birds. On investigating the cause of a serious epizootic among a group of young squabs, Beaudette (1926b) found *typhi-murium* in the heart-blood, internal organs and unabsorbed yolk of the young birds. The sick birds showed nervous symptoms, like incoordination of movements and convulsions, and diarrhoea; the lesions were swelling of the liver and lungs, catarrhal enteritis and inflammation of the proventriculus. The birds had been kept under very unhygienic conditions, which were regarded as a predisposing factor.

Several outbreaks of paratyphoid in pigeons from widespread areas in Germany were studied by Beck and Meyer (1927). The cause was ascribed to *typhi-murium* (Breslau) and the disease affected old birds as well as young ones. Beck (1929) considered that the etiological agent of pigeon paratyphoid resembled *typhi-murium* (Breslau) serologically, and that adult birds were much less susceptible than young ones and that the latter could be readily infected parenterally or otheriwse. Berge (1929) regarded paratyphoid as one of the most important diseases of pigeons in Germany —of 193 birds examined by him 22·6 per cent. were found to be

infected with *typhi-murium* (Breslau). Young birds commonly suffer from an acute form of the disease, but in older birds the condition is generally chronic and the symptoms may last for several weeks; the joints are swollen and there is paralysis of the muscles of locomotion and flight. Emmel (1929) also found *Schottmuller* (*typhi-murium?*) in practically pure culture in the exudates obtained from the swollen joints of pigeons examined by him.

By examining a flock of over 8,000 pigeons suffering from weakness of the wings and swelling of the joints Brunett (1930) found a straw coloured exudate in the joint swellings and abnormalities in the ovaries, resembling those of *pullorum* disease. *Typhi-murium* was isolated from the joints as well as from the ovaries.

Recently Jungherr and Wilcox (1934) investigated the cause of a disease in a flock of about 1,500 pigeons in which there was an annual loss of about 20 per cent. They incriminated an atypical non-maltose fermenting variant of *typhi-murium* as the etiological agent. In some cases *typhi-murium* could not be obtained from reacting squabs, while at other times the organisms were isolated from birds that failed to react serologically. Edwards (1935b) studied the same variant from three widely separated areas and found the " O " antigen, like that of *abortus equi*, lacking in factor V of the Kauffmann-White schema. The variant was noticed to be non-maltose fermenting and negative to the Bitter test; it appeared to be similar to *typhi-murium var. Copenhagen* of Kauffmann (1935a). Edwards labelled the organism *S. typhi-murium var. Storrs.* A similar organism, obtained from a case of purulent arthritis in a foal, is described by me above.

Lesbouyries and Verge (1932) described pigeon paratyphoid in France and Cernaianu and Popovici (1933) in Rumania, while Ismail Abu Bakr Khalifa (1935) studied an epizootic in Egypt due to *typhi-murium*. More recently Shirlaw and Ganapathy Iyer (1937) have recorded an outbreak of pigeon septicaemia in India caused by what they called a " Gaertner infection ". Soon after a number of birds had been inoculated with fowl-pox vaccine they developed symptoms of acute enteritis and fever from which they died. It is not possible to recognise the type of *Salmonella* incriminated from the description given.

That infection of pigeons with *Salmonella* may lead to serious outbreak of food-poisoning in man is illustrated by the description of Clarenburg and Dornickx (1932) of an epizootic which involved 20 persons in the military hospital at the Hague. The source of the infection was traced to pudding made largely from pigeons' eggs. *S. typhi-murium* was isolated from the pudding, and from the blood, faeces and urine of some of the patients; the sera of the affected persons also agglutinated cultures of the *Salmonella* found. On investigations, it was ascertained that the flock of pigeons from which the eggs originated were suffering from paratyphoid. Moreover, *typhi-murium* was recovered from eggs laid by these birds.

Although several outbreaks of a Septicaemic disease in pigeons have been reported in South Africa from time to time the cause has remained obscure until recently when Henning and Haig (1938)

studied an epizootic of squabs in which a *Salmonella* was found to be the cause. (The outbreak was studied after the completion of this paper.)

The affected birds suffered from loss of appetite, acute diarrhoea with green evacuations and rapid loss of condition. The most important lesions observed were enlargement of the spleen and liver and acute catarrhal enteritis. The affected flock was composed of over 200 birds, of which 24 have died from the disease. Heart-blood, spleen and liver cultures yielded a pure growth of a non-lactose fermenting, Gram-negative motile bacterium (culture 548). On testing this bacterium against various " O ", type and group sera of different groups of *Salmonella*, it was agglutinated by " O " sera containing factor IV of the Kauffmann-White Schema, by type sera containing factor *i* and by group sera. This suggested that the organism is related to *typhi-murium*. Agglutination and absorption tests were, therefore, performed with different varieties of *typhi-murium*. The results are given in Table 13.

The results of Table 13 show that *typhi-murium* absorbed all the agglutinins, " O " type and group, from 548 serum as well as from its own serum, but that culture 548 merely reduced the " O " titre of *typhi-murium* serum from 3,200 to 1,600. Culture 548 removed all the " O " agglutinins from its own serum but failed to exhaust a small portion of type and group agglutinins from both its own and *typhi-murium* serum. This is attributed to the reduction of its motility which occurs on subcultivation on solid agar.

When 548 serum was absorbed with either *typhi-murium* var. *Storrs* or *typhi-murium* var. *Copenhagen* all the " O " agglutinins were removed for *typhi-murium*, *typhi-murium* var. *Storrs*, *typhi-murium* var. *Copenhagen* and for itself.

According to these results, therefore, culture 548 contains the same type and group antigens as *typhi-murium* and the same " O " antigen as *typhi-murium* var. *Copenhagen* (Storrs). Its antigenic formula should be " O " = IV, type *i*, group 1, 2, 3.

An outbreak of pyo-arthritis in foals caused by the IV-variant of *typhi-murium* is described on page 124.

(2) Canaries.

Canaries seem to be particularly susceptible to *Salmonella* infection. They usually contract a very virulent form of the disease which may account for very severe losses in both young and adult birds. Joest (1906) was probably the first to draw attention to the occurrence of a disease in canaries caused by the enteric group of bacteria. Another early record of an epizootic apparently due to a *Salmonella* is that of Gilruth (1910). A bacterium isolated from the heartblood was found to be pathogenic for mice, rabbits, guinea-pigs and canaries. About the same time Pfeiler (1911) incriminated an organism of the *Paratyphi-B* group, obtained from blood culture, as the cause of a virulent outbreak of diarrhoea among a group of well-bred canaries. A somewhat similar outbreak was recorded by Lutje (1924).

TABLE 13.

Antigen.	548 Serum Unabsorbed.	*Typhi-murium* Serum Unabsorbed.	548 Serum* Absorbed by 548.	548 Serum Absorbed by *Typhi-murium*.	*Typhi-murium* Absorbed by 548.	548 Serum* Absorbed by *Typhi-murium* var. *Storrs*.	548 Serum Absorbed by *Typhi-murium* var. *Copenhagen*.
548—" O "	1,600	3,200	0	0	0	0	0
548 type	6,400	100,000	100	0	100	—	—
548 group	100,000	50,000	200	0	200	—	—
Typhi-murium—" O "	1,600	3,200	0	0	1,600	0	0
Typhi-murium type	6,400	100,000	100	0	100	—	—
Typhi-murium group	100,000	25,000	200	0	200	—	—
Typhi-murium var. *Storrs* " O "	1,600	—	—	—	—	0	0
Typhi-murium var. *Copenhagen* "O"	1,600	—	—	—	—	0	0

* Strain 548, although undoubtedly motile, lost a great deal of its motility when it was grown on solid agar. It, therefore, failed to absorb all the " H " agglutinins from its own serum as well as from *Typhi-murium* serum.

Later Beaudette (1926 a), Beaudette and Edwards (1926), and Harkins (1926) also described virulent epizootics in canaries in which organisms of the *Paratyphoid-B* group were incriminated as the etiological agent. Beaudette and Edwards (1926) studied two outbreaks in which birds of all ages were affected with severe diarrhoea associated with an increase in the amount of urates excreted; an organism which resembled *typhi-murium* serologically was obtained from the heart-blood and internal organs. The premises into which the birds studied by Harkins were introduced were well-kept and clean, and none of the local birds became affected; but the imported canaries arrived in soiled wooden cages, which probably played a predisposing part in setting up the infection.

In South Africa, Martinaglia (1929) recorded two outbreaks of paratyphoid in canaries in which *typhi-murium* was the cause, and in 1933 I investigated a very virulent epizootic in an aviary comprising about 200 well-bred canaries. The most important symptoms were drowsiness and diarrhoea, and the course of the disease was always very rapid, with a mortality of over 95 per cent. The most important lesions observed were hydro-pericardium, enteritis, tumor splenis and swelling of the liver. A gram-negative, non-lactose fermenting organism was obtained in pure culture from the heart-blood and spleen of all the birds examined. Cultures of this organism were readily agglutinated by *typhi-murium* serum and the organism was found to be di-phasic. A mixed serum was prepared by injecting a rabbit five times with a suspension of the canary strain (culture 176) in saline. Agglutination and absorption tests were then performed as shown in Table 14.

TABLE 14.

Antigen.	*Typhi-murium* S. a.b. *typhi-murium.*	*Typhi-murium* S. a.b. 176.	*Typhi-murium* S. unab.	176 S. a.b. *Typhi-murium.*	176 S. a.b. 176.	176 S. unab.
Typhi-murium " O ".....	0	0	1,600	0	0	800
Typhi-murium " H " type	0	0	50,000	0	0	12,800
Typhi-murium "H" group	0	0	25,000	0	0	6,400
176 " O "...............	0	0	1,600	0	0	800
176 type...............	0	0	50,000	0	0	12,800
176 group..............	0	0	25,000	0	0	6,400

S = serum; a.b. = absorbed by; unab. = unabsorbed:
0 = no agglutination at 1 in 100.

The results of Table 14 show that *culture* 176 removed all agglutinins (" O ", type and group) from *typhi-murium* serum as well as from 176 serum; while *typhi-murium* completely exhausted both 176 serum and the homologous serum. Culture 176, therefore, resembles *typhi-murium* serologically and contains the same antigenic factors; it should be regarded as *typhi-murium*.

On investigating the source of the infection, I found that losses commenced to occur soon after the owner had changed the food supply. Several samples of grain used by the owner for feeding were obtained and inoculated into enrichment media, e.g. tetrathionate broth. After 24 hours' incubation a loopful from each tube was spread on to a Mason tube of MacConkey's bile-salt agar. A few translucent, non-lactose fermenting colonies were observed on one of the tubes; some of these were picked and tested against drops of a *typhi-murium* serum dilution on a glass slide; the result was a coarse floccular agglutination and *typhi-murium* was suspected. The remainder of two of the positive colonies was subcultivated until a pure culture (*culture* 177) was obtained. Culture 177 was found to be diphasic, and, like *culture* 176, it exhausted all agglutinins from the serum of *culture* 176, as well as from the serum of *typhi-murium* Glasgow. Accordingly, *culture* 177, like *culture* 176, should be regarded as a strain of *typhi-murium*. A one-sided absorption was considered sufficient in the case of this test.

Although the presence of *typhi-murium* in the grain may explain the origin of the infection, the possibility of the grain becoming contaminated by attendants handling it after the outbreak among the canaries cannot be excluded. It may be of interest to mention that Jones and Wright (1938) described an outbreak of *typhi-murium* food-poisoning in man due to contamination of food with the excreta of mice.

Culture 153, obtained from one of a number of finches that were dying from a septicaemic disease in an aviary, was also studied. By testing it with the same method used for culture 176 it was found to be diphasic and to exhibit the same antigenic characters as cultures 176 and 177. It was, therefore, also labelled *typhi-murium*.

(3) Geese.

Outside Germany there is very little information available regarding the incidence of paratyphoid infection in geese. According to the classical monograph of Hubener (1910) seventeen outbreaks of food-poisoning traced to birds' meat have been recorded in Germany during the period 1903 to 1908; of these 14 outbreaks were due to goose meat, one to duck, one to fowl, and, in the case of one, the species of bird was not mentioned. Nine of the outbreaks were ascribed to paratyphoid organisms; of these, eight were caused by goose meat and one was due to the meat of a sick hen.

One of the first records of paratyphoid in geese is that of Pfeiler (1919) when he described a virulent epizootic among 9-week old birds with symptoms of septicaemia and swelling of the head and eyes. Heart-blood and organ cultures yielded a pure growth of an organism of the *Paratyphi-B* group (*typhi-murium?*). Earlier in the year cultures of *typhi-murium* were used for the eradication of mice and there was a suspicion that geese obtained the infection from the mice. Later Weissgerber and Müller (1922), Lutje (1924) and Burghoffer (1927) described similar epizootics among young geese. An organism which resembled both *Paratyphus-B* and *suipestifer* was obtained by Weissgerber and Müller from the heart-blood and

organs of affected birds, and a slow-growing *Salmonella* that was agglutinated to high titre by both *Schottmuller* and *Voldagsen* sera, was isolated by Lutje from the internal organs of diseased birds. Burghoffer investigated a septicaemic disease among 1 and 2 week old goslings and incriminated *Bact. enteritidis Breslau* (*typhi-murium*) as the causal agent. Apparently the young birds became infected after hatching as the blood of the laying hens gave a negative serological test with *typhi-murium*. Experimentally the bacterium isolated was found to be pathogenic for very young geese only, birds from 4 to 6 weeks old being completely refractory to artificial infection.

After the recognition of members of the genus *Salmonella* as etiological agents of disease in geese, several outbreaks of food-poisoning in man have been traced to goose meat or even to goose eggs. Thus Hohn and Becker (1927) reported a number of outbreaks of food-poisoning in man where foodstuffs, like salads and sausages, which contained either goose eggs or goose meat as ingredients, were incriminated. The symptoms in some of the cases resembled those of typhoid fever, while other cases were typical of *typhi-myrium* infection, with vomiting and diarrhoea as the chief symptoms. Baars (1929) also found *typhi-murium* as the cause of a disease in 12 persons that had partaken of some smoked goose breast. The organisms were isolated from the stools of the patients as well as from the suspected meat.

Later Baars (1931) described another outbreak of meat-poisoning in a family of three due to Breslau-infected goose meat. The meat was preserved in brine for a week before it was used. On investigation, he discovered that the goose from which the meat was obtained originated from the same farm as the birds that were responsible for the previous outbreak. Smoking and salting of the meat did not destroy the organisms, but rather caused their enrichment. Baars considered that freshly cooked or fried meats are less dangerous as the organisms are not very resistant to high temperatures.

Two outbreaks were recorded by Pressler (1930); the one involving four persons after a meal of pies that contained goose liver; *Breslau* (*typhi-murium*) was recovered from the stools of the patients and from what remained of the goose liver, but no infection could be detected in any of the remaining geese of the flock or in the persons that had handled the meat. The other outbreak affected a number of adults and a few children in a " Kinderheim "; they had eaten pies made from goose meat. During the same year Kolbe (1930) also described two epizootics of meat-poisoning resulting from the ingestion of goose meat.

On account of the increase in the number of cases of gastro-enteritis in man traced to goose meat, the carcasses of all suspicious-looking birds are now seized and condemned for human food in Germany. Out of 87 condemned carcasses of geese that had been suffering from fowl cholera, Hüsgen (1931) obtained *typhi-murium* from 11 and *enteritidis* from 1. Transportation of the birds was considered to reduce their resistance so that infection could readily

have taken place. Hüsgen also reported two outbreaks of food-poisoning due to goose liver and meat infected with *typhi-murium*, and in 1930 he investigated a severe epizootic of paratyphoid in geese.

During three months of 1932 Wundram and Schönberg (1932) examined 182 goose carcasses in Berlin and isolated *typhi-murium* from 44. The affected birds were emaciated and showed marked pathological changes in their internal organs, and their skins were reddened. The same workers also reported 6 outbreaks of food-poisoning, involving 16 persons, caused by goose meat and liver infected with *typhi-murium*. About the same time Bornstedt and Fiedler (1932) examined 828 geese imported from Poland and Lithuania; of these 182 had died and showed either lesions of fowl cholera or verminosis. Of 144 sick geese suffering either from transport injuries or symptoms of fowl cholera, 12 gave a positive agglutination reaction with *typhi-murium;* from the faeces of five of these *S. typhi-murium* was isolated. They suggested that *S. typhi-murium* probably occurs as a saprophyte in the bodies of geese, becoming invasive only when the animal's resistance has been lowered by factors like disease, injury and transportation.

As far as South Africa is concerned no cases of geese infected with *Salmonella* have so far been recorded.

(4) Ducks.

From the public health aspect *Salmonella* infection in ducks is particularly dangerous because the organisms may occur in the eggs of infected birds as well as in the meat. Moreover, paratyphoid is far more common in ducks and geese than in gallinaceous birds; Lecoq (quoted by Scott, 1930) considered the constant association of water birds with ponds and mud pools, which are sometimes contaminated with infected excreta, as the cause of the frequency of disease in them. In his account of paratyphoid infection in aquatic birds, Manninger (1918) described a disease in 1 to 2 weeks old ducks and geese caused by an organism of the *Paratyphus-B* group. Soon afterwards Rettger and Scoville (1920) investigated a most virulent disease ("keel") in ducklings; there was a mortality of nearly 100 per cent. in a flock of about 3,000, death usually occurring during the first week of life, but occasionally as late as 3 to 4 weeks after hatching. There were no definite lesions, but a *Salmonella* was readily obtained from the heart-blood and organs of the young birds and also from the ovaries of two adult ducks and the abdominal cyst of one. The investigators considered that the infection was probably transmitted from the ovaries of diseased hens through the egg to the chick, and they named the organism isolated *Bacterium anatum,* a new species. But Cooper and Krumwiede (1924), Edwards and Rettger (1924, 1927), and Kauffman and Silberstein (1934) found that only some of the strains of *Salmonella* labelled *anatum* could be included under the new name as the others resembled *typhi-murium* serologically; actually one of the strains studied by Kauffmann and Silbertstein (1934), strain 3123 of the National Collection of Type Cultures, was found to be *enteritidis*. *Anatum* like most other species of *Salmonella,* however,

affects more than one species of animal. Thus, Kauffmann and Silbertstein isolated a strain from the stool of a patient suffering from gastro-enteritis and intermittent fever, and another strain (*anatum var. Muenster*) from a person that had developed meat-poisoning after eating raw horse meat; they also described a third strain of human origin obtained from Kristensen. Edwards (1935 a) incriminated *anatum* as the etiological agent of an epizootic in chickens, and I (*vide infra*) isolated it from adult fowls.

Subsequently several different workers have recorded epizootics in ducks due to *Salmonellas* . Doyle (1927) recorded a severe outbreak among chicks and young ducks due to *typhi-murium*; the source of the infection remained obscure, but the food was suspected. In 1929 Gaiger and Davies (1930) investigated the first known outbreak of " keel " disease in Great Britain. There was a mortality of over 80 per cent. and the recovered birds remained ailing for several weeks. *Anatum* was obtained from a number of the birds examined. All the deaths occurred on a farm to which the young ducks were moved after hatching, while those that remained behind on the breeding farm remained healthy. It was apparent, therefore, that the infection took place after hatching and that the eggs and incubators were clean. Fermentation of the food was regarded as an important contributory factor in the genesis of the disease in this outbreak.

Pallaske (1930) described a disease in ducks associated with pathological changes in the ovaries of hens and the testes of drakes; the cause was found to be *S. enteritidis* Gaertner. Hole (1932) encountered three epizootics in young ducklings, one due to *enteritidis* and the other two to *typhi-murium*; infection was thought to have occurred through the egg. Acute and sub-acute enzootics in young ducks and geese with a mortality of 96 per cent. were described by Strozze (1931). Another virulent epizootic in ducklings with a death-rate of over 90 per cent. was recorded by Schaaf (1933). *Typhi-murium* was found to be the cause. Infected birds discharged the organisms with their faeces and gave positive agglutination reactions with these bacteria. Natural infection was thought to have resulted from the ingestion of food or water contaminated with infected excreta. Moreover, the vitality and resistance of the birds were considerably reduced by transportation over long distances by rail.

In England Dalling and Warrack (1932), McGaughey (1932), and Warrack and Dalling (1933) have shown that adult ducks may sometimes harbour *S. typhi-murium* or *S. enteritidis*, and that breeding birds with diseased ovaries are liable to lay infected eggs, which often fail to hatch; should the infected eggs hatch an epizootic of paratyphoid will probably occur among the newly-hatched birds. In this disease, therefore, as in Bacillary White Diarrhoea, the infecting agent is transmitted from the adult bird through the egg to its progeny. The presence of *Salmonellas* in the eggs laid by infected birds was demonstrated by these workers. Moreover, those ducks which laid eggs infected with either *typhi-murium* or *enteritidis* produced the corresponding agglutinins in their sera, and, as with *pullorum* infected hens, they could usually be detected by means of a serological test. Warrack and Dalling noticed that the eggs laid were infected only when the titre of the affected bird was high,

and the agglutination titre of the sera obtained from reactors dropped considerably during the course of the laying season. In the outbreak investigated by McGaughey, several deaths occurred among adult ducks during the course of months. The liver and ovary of one bird, which showed lesions resembling those of *pullorum* disease, yielded *enteritidis* on culture.

But healthy ducklings may acquire the infection from outside sources, e.g. infected eggs may introduce the infection into the incubator and so produce the disease in subsequent hatchings. Moreover, the infection may also be picked up from contaminated soil, food or water.

Scott (1930) considered that eggs may be responsible for many mysterious cases of *Salmonella* food-poisoning in which none of the common articles could be incriminated. He mentioned seven outbreaks where duck eggs were suspected, but not proved, to be the cause of the disease, and he alluded to a monograph of Lecoq (1906) in which several outbreaks of bacterial food-poisoning due to whipped cream were described; both duck and hen eggs were used as ingredients of the whipped cream. By dipping fresh eggs into a culture of *typhi-murium*, Scott showed that infection might pass through the shell, provided the eggs were kept in the room for at least two weeks; both yolk and albumen became infected. But he found that part of the shell must remain moist for the penetration of the bacteria; if the culture was allowed to dry on the shell, infection failed. The bactericidal action of fresh albumen prevented growth, but, as the eggs became stale, the multiplication of the *Salmonella* was marked and the eggs became badly infected. The infected eggs showed no outward sign of infection and might have been mistaken for normal eggs.

Later Scott (1932) described three widely-separated outbreaks of acute gastro-enteritis in man due to eggs infected with *typhi-murium*; there was one death. The organisms were recovered from the stools of a number of patients and from the organs of one. Duck eggs, fried and raw, were imputed and the suspicion was confirmed by the discovery of *typhi-murium* infected eggs from the corresponding flocks. The infected birds were recognised by means of serological tests and *typhi-murium* was isolated from the spleen, ovary, oviduct and intestines of some of the reactors.

Since the discovery by Scott and Dalling and Warrack of the transmission of *Salmonella* infection by means of duck eggs several cases have been revealed where foods containing infected duck eggs as ingredients have been incriminated as responsible for outbreaks of food-poisoning in man. Thus, Fromme (1933) and Willführ, Fromme and Bruns (1933) described 25 outbreaks of gastro-enteritis in Germany, traced to duck eggs infected either with *typhi-murium* or *enteritidis*; there were 143 cases and 2 deaths. In three of the outbreaks *Salmonellas* were discovered in the food, and in one it was possible to isolate *typhi-murium* from the faeces of two ducks and from the egg-shells of another. Furth and Klein (1933) recorded two epizootics of food-poisoning in large homes caused by vanilla pudding and potato salad containing duck eggs as ingredients; altogether 140 cases were involved. In one outbreak *typhi-murium*, and in the

other *Gaertner* bacilli, were isolated from the stools of the patients. The faeces of some of the ducks, from which the eggs for one of the establishments originated, yielded *aertrycke* on cultivation, but the examination of the contents of over a hundred eggs from a suspected flock failed to yield *Salmonellas*. These organisms were, however, obtained from the shells of three of the eggs examined. It was, therefore, thought that the infection was produced by the bacteria present on the shells. Müller and Rondenkircken (1933), on the other hand, obtained *Gaertner* bacilli in pure culture from the contents of the remainder of a consignment of duck eggs, some of which had been used in the raw state for a potato salad and were responsible for an outbreak of food-poisoning.

On investigating the cause of an epizootic of gastro-enteritis among a number of guests at a wedding party on a farm in Germany, Mieszner and Köser found that all the patients had partaken of a pudding made from duck eggs. From the ovaries of two ducks owned by the host, from an egg laid by one and from the faeces of another, *typhi-murium* was isolated. During the period 1931 to 1934 Bruns and Fromme (1934) studied 50 outbreaks of food-poisoning in Western Germany caused by foods containing duck eggs, prepared mostly in the form of mayonnaise. There were 253 cases and 6 deaths, and either *typhi-murium* or *enteritidis* was incriminated. Zeug (1935) also drew attention to the increasing prevalence of food-poisoning in the industrial areas of Western Germany due to foods prepared from duck eggs; mayonnaise, potato salads, puddings and Hackfleisch were most frequently responsible. *Typhi-murium* was regarded as the chief cause. Zeug has pointed out that, although no definite clinical symptoms may be observed in the birds that lay infected eggs, egg-laying generally decreases, and pathological changes develop in the ovaries and oviducts. *Salmonellas* are usually present in these lesions, from which they find their way into the interior of the egg. But infection sometimes occurs by contamination of the egg-shell with infected faeces. As shown by Scott (1930—*vide supra*), *Salmonellas* may penetrate through the shell into the interior of the egg, under certain conditions. If the shell-contaminated eggs are soon cooked, no harm is likely to result; but should they be kept for some time, serious infection may follow their use. The heating to which eggs are generally subjected is not enough to destroy the organisms present in an infected egg. After 5½ minutes boiling infected eggs may still contain live organisms, but 6 minutes boiling is usually sufficient to kill all the bacteria.

Clarenburg and Pot (1935) also described a severe outbreak of gastro-enteritis in 4 families, involving 9 persons. Symptoms of diarrhoea, vomiting and fever appeared soon after the people had eaten cream puffs supplied by the same baker. *Typhi-murium* was isolated from the cream puffs, and from the stools and urine of some of the patients. Duck eggs were used as ingredients of the puffs, but all the eggs examined from the suspected ducks gave negative results for *Salmonella*. Six of the ducks, however, gave positive serological tests for *typhi-murium*, and this organism was isolated from the faeces of one. When the reacting ducks were killed, they showed lesions of chronic oophoritis, and from the ovaries of two of

them *typhi-murium* was obtained in pure culture. Similar bacteria were also isolated from apparently normal looking yolks present in the ovaries.

Recently epizootics in ducks, due to infection with either *Gaertner* or *typhi-murium*, have been observed fairly frequently in Holland, where the disease has been studied by a number of investigators, especially Jansen (1934a, 1934b, 1935, 1936). In a virulent outbreak among young ducklings with lesions of enteritis and swelling of the liver, he isolated *enteritidis* bacilli of the Moscow type from the internal organs of affected birds. By testing a number of suspected birds serologically, he found a few affected with oophoritis in which the reaction was negative, though in some positive cases there was no evidence of oophoritis. Generally, however, the ovary was affected when a positive reaction had been obtained.

Jansen has also noticed that a large percentage of ovary-infected ducks lay infected eggs, which frequently cause epizootics of paratyphoid among newly hatched ducklings. But he has also recorded a number of outbreaks in young birds where the eggs could not be incriminated.

In five outbreaks studied by Jansen in 1936, three were found to be due to *enteritidis var. Essen*, one to *typhi-murium* and one to a mixture of the two organisms. In the latter case *typhi-murium* was obtained from the heart-blood, liver and yolk of the young birds, while a small percentage of the adults was infected with *essen* as well as *typhi-murium*.

The importance of *Salmonella* infection in both ducks and geese in Germany was also emphasised by Lerche (1936), who found 5·7 per cent. of the duck's eggs offered for sale to be infected. He described an outbreak of food-poisoning in a family that had eaten fried ducks eggs. *Typhi-murium* was isolated from the stools of the patients and from the eggs.

I have not had an opportunity of studying *Salmonella* infection of ducks in South Africa, but in 1931 Dunning (1934) investigated a virulent epizootic of ducklings in the Cape Peninsula. At least 50 per cent. of a flock of 2,000 birds died at ages varying from 5 to 23 days. "Keel" disease was tentatively diagnosed. Fourteen newly hatched ducklings taken from the infected farm were removed to fresh, clean premises and kept under observation. All died from 5 to 29 days after hatching. A bacillus obtained in pure culture from the organs of affected birds was tested biochemically and was found to react like *S. enteritidis*. The evidence collected by Dunning suggested that the eggs were infected at the time they were placed in the incubator.

Early in 1932 Coles also investigated a virulent epizootic of ducklings in the Transvaal, and isolated a Gram-negative, non-lactose fermenting bacterium from the affected birds. Fermentation tests carried out with this organism resembled those obtained with *typhi-murium*.

As the cultures made from the organisms isolated from both outbreaks were discarded, serological tests could not be performed.

(5) Turkeys.

About 45 years ago MacFadyean (1893) described a disease in turkeys which he called " epizootic pneumo-pericarditis ". The organism obtained by MacFadyean from the heart-blood, spleen and pericardium is probably a *Salmonella*, and the outbreak of " pneumo-pericarditis " caused by it is the first record of paratyphoid among turkeys. The etiology of the disease " pneumo-enteritis ", described by Dodd (1905), is less apparent. The organism incriminated was a non-motile bacterium of the " fowl-cholera " type, obtained in pure culture from the heart-blood and lungs. In South Africa, Jowett (1908) investigated a highly fatal disease in turkeys, which he also called " pneumo-pericarditis " after the condition described by MacFadyean. Cultures of the organism isolated from the heart-blood and pericardial fluid proved to be pathogenic for turkeys and guinea-pigs, but not for fowls. It is highly probable that Jowett was also dealing with an outbreak of paratyphoïd. However, the first authentic record of an epizootic in turkeys, in which a *Salmonella* was recognised as the causal agent, is that of Pfaff (1921). A pure culture of a paratyphoid-like organism was isolated from the heart-blood and pericardial fluid of diseased birds. Cultures of this bacterium proved to be pathogenic for turkeys and several small laboratory animals.

Later, several other investigators studied outbreaks of paratyphoid in turkeys. Rettger, Plastridge and Cameron (1933) investigated outbreaks of recurrent deaths among young poults on two different farms; the greatest losses occurred among birds that were less than 10 days old, but deaths were also observed as late as 6 weeks after hatching. A pure culture of *typhi-murium* was obtained from the heart-blood and internal organs and it was thought that the unhygienic conditions under which the birds were kept on the one farm accounted for the ease with which the disease became established.

According to Lee, Holm and Murray (1936) no serious losses were known to occur in turkeys in the State of Iowa prior to 1934. In May of that year a very virulent disease, with a mortality of over 90 per cent., appeared in young poults under 5 weeks old. A pure culture of *typhi-murium* was obtained from the heart-blood and internal organs of affected birds. More recently Cherrington Gildow and Moore (1937) investigated four outbreaks of *typhi-murium* infection among poults in widely separated areas. In three of the outbreaks the disease appeared before the birds were a week old, suggesting that the infection was probably transmitted, like *pullorum* disease, from infected hens through the eggs to the poults. A large percentage of the hens that produced diseased poults gave positive agglutination reactions with *typhi-murium*. There was a mortality of over 80 per cent. among the poults under 10 days of age. In one outbreak *typhi-murium* was isolated from some dead-in-the-shells poults, and in another from the ovaries and yolk of some of the reacting hens; in some cases, however, no organisms could be cultivated from the abnormal ovaries of reacting hens.

But outbreaks of paratyphoid in turkeys may be caused by *Salmonellas* other than *typhi-murium*. Edwards (1937) has described an epizootic in poults due to *S. senftenberg*, the fist record in which this organism has been incriminated as the cause of an animal disease. Moreover, infection of turkeys with *S. gallinarum* is comparatively frequent. Two of the 149 outbreaks caused by this bacterium and recorded by me (*vide infra*) involved turkeys only.

(6) Fowls.

Virulent epizootics, like fowl-typhoid and bacillary white diarrhoea, are so common in gallinaceous birds that it is quite possible that some outbreaks due to other types of *Salmonella* have been mistaken for these diseases. In many outbreaks of paratyphoid the scourge affects only very young birds in the same way as *pullorum* disease attacks eggs laid by infected hens, while in other epizootics older birds also suffer severely, and the disease resembles fowl typhoid. The nature of the malady is revealed only when a careful bacteriological examination of the dead birds is made.

One of the first records of a disease that can be interpreted as paratyphoid in fowls is the description by Mazza (1899) of an epizootic among these birds in Italy. A motile, non-indol forming, glucose fermenting organism obtained from the internal organs was found to be pathogenic for fowls and pigeons, but not for rabbits.

But there are very few early descriptions of paratyphoid in fowls, probably on account of the marked resistance of adult birds to infection. Reinholdt (1912) and others have tried to infect fowls, geese, ducks and pigeons with *enteritidis* and *paratyphoid-B* (*typhi-murium?*) both parenterally and per os; fowls proved to be the most resistant. Pfeiler and Rehse (1913) also found that fowls were not very susceptible to paratyphoid infection. They studied the outbreak on a farm of a chronic disease which occurred enzootically with a few deaths reported from time to time and recovered a bacterium from the internal organs of affected birds which they placed in the *Paratyphus-B* group. Nevertheless, although adult fowls may not be very susceptible to natural or artificial infection with certain types of *Salmonella*, numbers of very virulent outbreaks of paratyphoid in chickens are reported periodically. Thus Spray and Doyle (1921) found that outbreaks of a very destructive disease in newly hatched chicks (2 to 4 days old) may be caused by organisms of the paratyphoid-B group, as well as by *S. pullorum*. Edwards (1929) investigated an epizootic affecting over 2,000 very young chicks with a mortality rate of about 25 per cent. *S. pullorum* could not be detected in any of the birds examined, but there was a mixed infection of *typhi-murium* and *anatum* associated partly with coccidiosis. By means of serological tests and post mortem examinations, no carriers could be detected in either the breeding stock or in the survivors.

Later five separate outbreaks of paratyphoid in birds were recorded by McGaughey (1932). Of these three occurred in chicks, one in adult fowls and one in ducks. In one of the chicken epizootics *typhi-murium* was obtained from the heart-blood and internal organs of the dead birds; in another outbreak a non-motile

strain of the *typhi-murium Paratyphi-B* group was isolated from the carcasses, while *enteritidis* was recovered from the third group of chickens. The disease in the adult fowls caused a large number of sudden deaths. McGaughey isolated *typhi-murium* from the internal organs of one bird and *pullorum* from another. In the outbreak affecting the ducks several deaths occurred during the course of a few months. The ovary of one of the ducks examined resembled that of a case infected with *pullorum*, and *S. enteritidis* was obtained from its liver and ovary. It was stated above that Jansen (1936) isolated *enteritidis var. Essen* from the organs of diseased ducks, and also from the yolk sac of chickens that had been living in close association with ducks.

During the course of the routine diagnosis of chick disease, Jungherr and Borden (1934) encountered 5 cases of paratyphoid infection. In two of these the causal agent was found to be *typhi-murium var. storrs*, in two atypical strains of *cholerae-suis*, and in one an atypical strain of *L2*.

In spring of 1936 Schalm (1937) investigated a *pullorum*-like disease that affected several batches of chicks on a Californian farm. The breeding stock had been healthy for a number of years and the farm was free from bacillary white diarrhoea, but deaths were reported in the chicks sold to five different farmers, and about 40 per cent. losses were sustained in 4 to 10 days old birds. *Typhi-murium* was isolated from the heart-blood and organs, and chilling during shipment was considered to be a predisposing cause. The chicks that remained on the breeder's farm developed an apparently chronic form of the disease which affected fewer and much older birds. It was thought that infection of the chickens on the farm took place in the incubator after hatching by means of bacteria present in the faecal matter on the surface of the egg shells. Schalm could not infect 4-day old chickens either by feeding or by intravenous inoculations of cultures of *typhi-murium*.

According to Emmel (1936) different species of *Salmonella* may occur as facultative parasites in the alimentary canal of fowls; by examining the intestinal contents of a number of fowls suffering from enteritis due either to coccidiosis or to worm infestation he claims to have isolated *aertrycke*, *paratyphi-A*, *paratyphi-B*, *enteritidis*, *typhi* as well as *pullorum*. The account published by Emmel does not appear to be complete; there are no records given of the methods used for typing the strains, and it is not stated on what grounds the different strains were classified. To me it seems that Emmel's claims cannot be accepted, unless much more information is available than is presented in his report.

The incidence of food poisoning produced by fowl's meat is apparently much lower than that caused by foods prepared from duck and goose meat and eggs. In a review of outbreaks of food-poisoning due to bird meat, Beller (1933) discussed several cases where the meat of aquatic birds was incriminated; he pointed out that fowl and pigeon meat intended for food are always well cooked so that food-poisoning cannot be readily set up even when the meat is infected. From 1923 to 1932 Meyer (1933) studied 50 outbreaks of food-poisoning caused by bird meat. Three hundred people were

affected and there were three deaths; 37 of the outbreaks were due to goose, 3 to duck, 7 to fowl, one to partridge, one to pigeon meat and in one case both goose and fowl meat were incriminated. Although the type of *Salmonella* recovered was not determined in all outbreaks, *typhi-murium* was found to be by far the most common; *enteritidis* was incriminated in a small number of the cases, while "*Paratyphus-B*" and a *newport*-like organism were recovered from one outbreak. The bacteria were generally isolated either from the suspected food, or from the patients, or from both food and patients. As stated above, the importance of ducks as carriers of *Salmonella* infection lies rather in the eggs than in the meat.

Although *S. gallinarum* and *S. pullorum* are generally regarded as non-pathogenic for man, Kauffmann (1934) has described a strain of *gallinarum* (the Duisberg strain) which he isolated from the stools of patients that developed acute symptoms of gastro-enteritis after they had partaken of a salad. The organism resembled *gallinarum* serologically, and was pathogenic for chickens, but its fermentation reactions were atypical.

Apart from infection with *gallinarum* and *pullorum*, I have studied four outbreaks of *Salmonella* infection in fowls in South Africa.

I. The information relating to this outbreak has been furnished by me in another paper (Henning, 1937).

In 1935 a farmer at Amersfoort in the Transvaal sustained serious losses amongst his chickens from what appeared to be an infectious disease. The disease was not investigated and the cause of the mortality remained unknown until the end of 1936, when the malady reappeared and a few affected birds were sent to Onderstepoort for examination. An apparently pure culture, obtained by Mr. J. D. W. A. Coles, head of the Poultry Disease Section, from the heart blood of a 7-day-old chick, was handed to me for identification. The culture was plated and a few isolated colonies were picked. The cultures obtained from these were tested against various agglutinating sera. It was found that the antigenic structure of the organism exhibited an entirely new combination of antigenic components; for this reason, therefore, the germ should be admitted to species rank in compliance with the recommendations of the Salmonella Sub-committee of the Nomenclature Committee of the International Society of Microbiology (1934). The name *Salmonella amersfoort* (Henning, 1937) was given to the organism—after the place of its origin.

Morphology and cultural character.—Morphologically, *S. amersfoort* resembles a typical *Salmonella*, and, like it, grows readily on ordinary laboratory media. It is Gram-negative and actively motile. Saline and thermo-agglutination tests, as well as the shape of individual colonies, show that it is smooth.

Biochemical character.—*S. amersfoort* forms acid and gas in glucose, dulcite, mannite, maltose, arabinose, rhamnose, and sorbite; it forms hydrogen sulphide and renders litmus milk alkaline; it does not produce indol.

Pathogenicity.—*S. amersfoort* is pathogenic for chickens and mice; 0·25 c.c. of a 24-hour-old broth culture, given intraperitoneally, kills a 6-week-old chicken in 4 days and 0·05 c.c. kills an adult mouse in 36 hours. *S. amersfoort* was recovered from the heart blood and spleen in each case. But chickens dosed with 1 c.c. of the virulent broth culture remained apparently healthy.

While making an antigenic analysis of *Salmonella amersfoort* I noticed well-marked flocculation occurring between this organism and the sera of organisms that are generally regarded as not even remotely related antigenically.

Bruce White (1929) described three forms of antigenic variation occurring in the genus *Salmonella*: (1) the " H " form—" O " form variation of Weil and Felix (1920), (2) the Smooth form—Rough form variation of Arkwright (1921), and (3) the specific phase—non-specific phase variation of Andrewes (1922). Later Kauffmann and Mitsui (1930) described a new type of phase variation, involving the specific phases of *brandenburg*, *dar-es-salaam* and *Potsdam*; and they called this $\alpha - \beta$—variation. A similar variation has been observed in a number of other types of *Salmonella*, viz. *abortus-bovis* (Bernard, 1935), *hvittingfoss* and *oslo* (Tesdal, 1936, 1937), *bispebjerg* and *typhi* (Kauffmann, 1936a, 1936b), *chester* and *schleissheim* (Kauffmann and Tesdal, 1937). The antigenic structure of these organisms, according to Kauffmann and Tesdal (1937), is given in Table 20B. The inagglutinable (containing Vi antigen) and agglutinable forms of *S. typhi* described by Felix and his co-workers (1934, 1935, 1936) may be regarded as another type of variation. Kauffmann (1935) introduced the terms " V-form " and " W-form " to denote, respectively, the variant containing Vi-antigen and that devoid of it; while Craigie and Brandon (1936), Brown (1936), Scholtens and others showed the effect of bacteriophage on the V – W degradation.

Serology.—For the study of the antigenic structure of *S. amersfoort*, " O " sera, " H " specific and non-specific sera, and mixed " O " and " H " sera, prepared against a number of representative strains of *Salmonella*, were used. Sera prepared against *S. amersfoort* were also used. The sera and agglutinating suspensions were prepared according to the methods described above.

Preliminary tests showed that *amersfoort* gave a well-marked fine granular agglutination with " O " sera containing factors VI and VII of the Kauffmann-White schema (*cholerae-suis*, *newport*, *potsdam* and others), while a distinctly coarse floccular agglutination was produced not only with " H " sera containing factors *en* or *e* (*abortus equi*, *brandenburg*, *potsdam*, *dar-es-salaam*, *onderstepoort*, *newport*, *reading* or *anatum*), but also with those containing factor *d* (*stanley*, *muenchen* and *typhi*). However, a much stronger agglutination was produced by sera containing factors *en* than with those containing factor *e* but not *n*.

The culture was again plated on Mason tubes to obtain a number of separate colonies for independent study. After 5 hours' incubation at 37° C. broth cultures of these colonies were tested against *Kunzendorf* and *Binns* group sera as well as against the type sera of

onderstepoort, *newport*, *potsdam* and *typhi*. The results are given in Table 15. It will be noticed that the majority of the cultures agglutinated with *typhi* serum (factor *d*), a number agglutinated with *potsdam* (factors *enlv*), and *newport* (factors *eh*) or *onderstepoort* (factors *eh*) sera, a few agglutinated incompletely with all four sera, being apparently intermediate forms, but no agglutination whatsoever was effected with *Kunzendorf* and *Binns* sera.

TABLE 15.

Thirty colonies grown in broth for 5 *hours and tested against* 5 *different sera.*

No. of Colony.	*Typhi* s.	*Newport* or *Onderstepoort* s.	*Potsdam* s.	*Kunzendorf* s.	*Binns* s.
1–16............	++++	0	0	0	0
17–25............	0	++++	++++	0	0
26–30............	+	+	+	0	0

++++ = complete flocculation within 30 minutes.
+ = partial flocculation after 1 hour.
0 = no flocculation after 18 hours.
In headings to table s. = serum.

These results indicated (1) that the organism occurred only in the type phase and (2) that the culture used was either a mixed one or that it exhibited properties that have hitherto not been described in a member of the *Salmonella* group. In order to settle the matter of the purity of the strain, Dr. J. H. Mason kindly single-celled fresh cultures derived from a colony of each of the two types—i.e. from one colony agglutinating only with sera made against specific factor *d* and from another that flocculated solely with the anti-sera of specific factors *en* and *eh*. After plating the primary cultures obtained from

TABLE 16A.

Twenty-two colonies picked from the plate seeded with growth from the single cell obtained from colony 1, *Table* 15.

No. of Colony.	*Typhi* Serum.	*Onderstepoort* or *Potsdam* Serum.
1 to 21....................................	++++	0
22..	0	++++

the single cells a number of well-isolated colonies were again picked into broth tubes and incubated at 37° C. for 5 hours—in order to reduce the lag phase in the growth the broth tubes were placed in a water-bath at 40° C. for 10 minutes before transferring them to the incubator.

Four single cells (*a b c* and *d*) obtained from colony 1, Table 16B, were now cultivated separately in broth and plated. A number of colonies from each plate were picked into broth, incubated and tested against both *d* and *en* sera. The results are given in Table 17.

TABLE 16B.

Thirty colonies picked from the plate seeded with the broth culture from single cell of colony 17, *Table* 15.

No. of Colony.	*Typhi* Serum.	*Onderstepoort* or *Potsdam* Serum.	Saline Control.
1 to 28	0	++++	0
29 and 30	++++	0	0

TABLE 17.

Single Cell.	No. of Colony.	*Typhi* Serum.	*Onderstepoort* or *Potsdam* Serum.
a	1 to 4	++++	0
a	5 to 12	0	++++
b	1 to 14	0	++++
b	15	++++	0
c	1 to 14	++++	0
c	15	0	++++
d	1 to 10	++++	0

++++ = complete flocculation after 30 minutes.
0 = no flocculation after 18 hours.

Therefore, these results clearly show that *S. amersfoort* is composed of two distinct "H" antigenic complexes, both of which occur in the specified phase; the second (*d*-) antigen, apparently corresponding to the α phase of Kauffmann and Mitsui (1930), is agglutinated with the "H" serum of *typhi* and, as will be shown below, also with specific sera of other *Salmonellas*, *stanley* and *muenchen*, containing specific factor *d*, while the other component, the first (*en*-) antigen, apparently corresponding to the β phase of Kauffmann and Mitsui (1930), is agglutinated solely with *potsdam*, *onderstepoort*, and other sera containing aglutinins for the type factors *en* and *eh* (*vide infra*). Sera containing agglutinins for

factors *en* always give a much stronger flocculation than the anti-sera of factors *eh*. It has also been shown that single cells composed of either the one or other complex constantly give rise to daughter cells some of which resemble the parent cell antigenically, while others have adopted a new antigenic structure entirely different from that present in the parent. The latter daughter cells again give rise to offspring some of which resemble themselves, while others are like the parent. These mutations constantly proceed and cells containing either the one or other antigenic complex continually produce cells of both types, and neither the one nor the other type of cell has been found to breed entirely true.

On single-celling the growth obtained from each of the two types of colonies serially three successive times, both variants constantly appear in the cultures arising from the single cells.

The purity of the culture is therefore beyond dispute; it is the property of the bacterium of giving rise to two distinct types of variants in the specific phase that is responsible for the uncommon behaviour of the culture. The organism apparently does not occur in the non-specific phase.

" O " agglutination.—Cross-agglutination tests were carried out with the heat-stable " O " antigens and " O " sera of the different *Salmonella* type of the Kauffmann-White schema; also with *S. aberdeen* (Smith, 1934), *S. poonae* (Bridges and Scott, 1935) and *S. onderstepoort* (Henning, 1936). The reactions are given in Table 18.

TABLE 18.—*" O " Agglutination.*

	" O "—ANTIGEN.				
	Amersfoort.	*Potsdam.*	*Muenchen.*	*Onderstepoort.*	*Brandenburg.*
UNABSORBED SERA—					
Amersfoort s.	800	800	200	100	0
Potsdam s.	800	800	—	—	—
Muenchen s.	200	—	1,600	—	—
Brandenburg s.	0	—	—	—	1,600
Onderstepoort s.	50	—	—	800	—
ABSORBED SERA—					
Amersfoort s.a.b. Amersfoort	0	0	—	—	—
Amersfoort s.a.b. *Potsdam*..	0	0	—	—	—
Amersfoort s.a.b. *Muenchen*.	200	—	0	—	—
Amersfoort s.a.b. *Brandenburg*	800	—	—	—	0
Potsdam s.a.b. *Potsdam*....	0	0	—	—	—
Potsdam s.a.b. *Amersfoort*..	0	0	—	—	—
Muenchen s.a.b. *Amersfoort*.	0	—	800	—	—

0 = less than 1 : 50. — = not tested.

In this table s. = serum; s.a.b. = serum absorbed by.

The results show that *amersfoort* " O " as well as *potsdam* " O " sera are completely exhausted for the homologous " O " antigen by *amersfoort*. In the same way both sera are exhausted by *potsdam*. The somatic " O " antigen of *amersfoort* must, therefore, be regarded as identical with that of *potsdam*, i.e., it is composed of factors VI, VII.

" *H* " *agglutination.*—Flocculation, approximately equivalent in titre to that produced with the homologous antigen, was obtained with the specific sera of *abortus equi*, *potsdam*, *brandenburg*, *dar-es-salaam*, *muenchen* and *typhi*, but a much weaker agglutination resulted when the type of serum of *onderstepoort*, *newport*, *reading* or *anatum* was used for the test. In the same way *amersfoort* " H " serum agglutinated the specific antigens of *abortus equi*, *potsdam*, *brandenburg*, *dar-es-salaam*, *stanley*, *muenchen* and *typhi* almost up to full titre, while its titre for type antigens containing factors *eh* was much lower.

On absorbing *amersfoort* " H " serum with the specific phase of either *potsdam* (factors *enlv*), *brandenburg* (factors *enlv*) or *dar-es-salaam* (factors *enlw*) the titre of the serum for one of the homologous specific antigens (*en*-), β phase, was reduced from 6,400 to approximately 800, while the titre for the other homologous specific antigen (*d*-), α phase, as well as for *stanley*, *muenchen* and *typhi* (factor *d*) remained unaltered. When *abortus equi* (factors *enx*) was used for the absorption, the reduction in titre for the homologous *en* antigen (β phase) was almost complete, but still no noticeable decrease in agglutinins for the homologous *d* antigen (α phase) was effected; a small residue, however, remained which caused an incomplete agglutination with the *en*-variant (β phase) of *amersfoort*. The cause of this flocculation is discussed below.

On the other hand, when *amersfoort* " H " serum was absorbed with either *stanley*, *muenchen* or *typhi* (factor *d*) most of the agglutinins for the one variant (*d*-) α phase, of *amersfoort* were removed, while the titre for the other homologous antigen (*en*-) β phase, remained unaltered (Table 19).

When either *potsdam* or *brandenburg* serum was absorbed with *amersfoort*, all agglutinins for *amersfoort* were removed, but the titre of the serum for *panama* (factors *lv*) and *london* (factors *lv*) was not affected. Moreover, the treated serum still agglutinated the homologous antigen although the flocculation was incomplete and the fluid remained turbid, due, no doubt, to the persistence of *lv* agglutinins in the serum. *Dar-es-salaam* serum behaved in practically the same way, but *amersfoort* is apparently capable of removing all the agglutinins for the homologous antigen from *abortus-equi* serum.

On absorbing either *stanley*, *muenchen* or *typhi* serum with *amersfoort*, most of the agglutinins for the homologous " H " specific antigen were exhausted, *muenchen* serum being exhausted much more completely than either *stanley* or *typhi* serum, while all the agglutinins for the second variant of *amersfoort* (factor *d*-) were removed.

TABLE 19.

"H" SPECIFIC ANTIGEN.

	Amersfoort d.	*Amersfoort en.*	*Typhi.*	*Stanley.*	*Muenchen.*	*Potsdam.*	*Brandenburg.*	*Dar-es-salaam.*	*Abortus-equi.*	*Onderstepoort.*	*Newport.*	*Reading.*	*Anatum.*	*Panama.*	*London.*
UNABSORBED SERA.															
*Amersfoort d.*s.	12,800	6,400	12,800	12,800	12,800	1,600	3,200	3,200	3,200	200	—	—	—	0	0
Amersfoort en. s.	6,400	6,400	6,400	6,400	6,400	3.200	6,400	3,200	6,400	400	400	400	400	0	0
Typhi s.	12,800	0	12,800	12,800	12,800	—	—	—	—	—	—	—	—	—	—
Stanley type s.	25,600	400	25,600	25,600	25,600	—	—	—	—	—	—	—	—	—	—
Muenchen type s.	6,400	0	6,400	6,400	12,800	—	—	—	—	—	—	—	—	—	—
Potsdam s.	0	6,400	—	—	—	6,400	6,400	6,400	3,200	800	—	—	—	6,400	1,600
Brandenburg s.	0	6,400	—	—	—	6,400	12,800	3,200	3,200	400	—	—	—	6,400	3,200
Dar-es-salaam s.	0	1,600	—	—	—	—	—	3,200	—	—	—	—	—	1,600	—
Onderstepoort s.	0	400	—	—	—	200	—	200	200	1,600	—	—	—	—	—
Abortus-equi s.	—	6,400	—	—	—	1,600	3,200	6,400	3,200	—	—	—	—	—	—
ABSORBED SERUM.															
Amersfoort d. s.a.b. *Amersfoort*	0	0	0	0	0	0	—	—	0	—	—	—	—	—	—
Amersfoort d. s.a.b. *Typhi*	400	6,400	0	—	—	—	—	—	—	—	—	—	—	—	—
Amersfoort d. s.a.b. *Stanley*	400	3,200	—	0	—	—	—	—	—	—	—	—	—	—	—
Amersfoort d. s.a.b. *Muenchen*	400	3,200	—	0	0	—	3,200	3.200	—	—	—	—	—	—	—
Amersfoort en s.a.b. *Potsdam*	6,400	800*	—	—	—	0	—	0	800	—	—	—	—	—	—
Amersfoort en s.a.b. *Brandenburg*	6,400	800*	6,400	—	—	—	0	—	—	—	—	—	—	—	—

0 = less than 1 : 50. * = partial flocculation, fluid remaining turbid.
In this table s. = serum, s.a.b. = serum absorbed by. — = not tested.

TABLE 19 (*continued*).

	Amersfoort d.	*Amersfoort* en.	*Typhi.*	*Stanley.*	*Muenchen.*	*Potsdam.*	*Brandenburg.*	*Dar-es-salaam.*	*Abortus-equi.*	*Onderstepoort.*	*Newport.*	*Reading.*	*Anatum.*	*Panama.*	*London.*
ABSORBED SERUM (*continued*).															
Amersfoort en s.a.b. *Dar-es-salaam*	6,400	1,600*	—	6,400	—	—	—	0	—	—	—	—	—	—	—
Amersfoort en s.a.b. *Abortus-equi*	6,400	200	—	—	—	—	0	—	0	—	—	—	—	—	—
Typhi s.a.b. *Amersfoort*	0	0	800	—	—	—	—	—	—	—	—	—	—	—	—
Typhi s.a.b. *typhi*	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—
Stanley s.a.b. *Amersfoort*	0	0	—	400	400	—	—	—	—	—	—	—	—	—	—
Stanley s.a.b. *Stanley*	0	0	—	0	—	—	—	—	—	—	—	—	—	—	—
Muenchen s.a.b. *Amersf.*	0	0	—	0	100	—	—	—	—	—	—	—	—	—	—
Potsdam s.a.b. *Amersf.*	—	0	—	—	—	6,400*	6,400	6,400	0	—	—	—	—	6,400	3,200
Brandenburg s.a.b. *Amersfoort*	—	0	—	—	—	—	6,400	—	—	—	—	—	—	6,400	3,200
Dar-es-salaam s.a.b. *Amersfoort*	—	0	—	—	—	800	—	1,600	—	—	—	—	—	1,600	800
Abortus-equi s.a.b. *Amersfoort*	0	0	—	—	—	0	0	—	0	—	—	—	—	—	—
Potsdam s.a.b. *Amers.* and then by *Panama*	—	—	—	—	—	100	100	—	—	—	—	—	—	0	0
Brandenburg s.a.b. *Amersfoort*, then by *London*	—	—	—	—	—	—	200	—	—	—	—	—	—	0	0
Abortus-equi s.a.b. *Potsdam*	—	400	—	—	—	—	0	—	200	—	—	—	—	—	—

0 = less than 1 : 50. * = partial flocculation, fluid remaining turbid.
In this table s. = serum, s.a.b. = serum absorbed by. — = not tested.

Discussion.

These results show that *Salmonella amersfoort* contains two distinct antigenic complexes, the one, β phase, corresponding to factors *en* of *abortus-equi, potsdam, brandenburg* and *dar-es-salaam* plus an additional factor, part of which apparently corresponds to factor *x* of *abortus-equi*; the other complex, α phase, coincides largely with factor *d* of *stanley, muenchen* and *typhi*. The additional factor is probably responsible for the residue of agglutinins left for the first (*en*-) antigen, β phase, after absorbing *amersfoort* serum with *potsdam, brandenburg* or *dar-es-salaam;* but, although factor *x* of *abortus-equi* apparently forms a part of this additional factor, there may be another component which is not present in *abortus-equi*. The fact that *amersfoort* exhausts all agglutinins from *abortus-equi* serum for itself as well as for the homologous specific antigen indicates that *amersfoort* contains all the specific antigenic components of *abortus-equi*, i.e. factors *enx*; but since *abortus-equi* fails to exhaust *amersfoort* serum completely for the homologous first (*en*-) antigen it is possible that this antigen of *amersfoort* contains a minor factor in addition to the *enx* of *abortus-equi*.

After absorbing *amersfoort* serum with either *stanley, muenchen* or *typhi*, a small residue is left which still agglutinates the homologous second (*d*-) antigen, α phase, but not the specific antigen (factor *d*) of either *stanley, muenchen* or *typhi*. It is not quite clear to what this residue can be ascribed; whether it should be regarded as an extra factor in the second (*d*-) antigen, α phase, in addition to factor *d* of *stanley, muenchen* and *typhi*, or whether it can be attributed to a trace of the first (*en*-) antigen, β phase, present in the emulsion of the second (*d*-) antigen, α phase, of *amersfoort* used for the test, is not certain. If the latter explanation holds it is likely that the agglutination occurring in *amersfoort* serum absorbed with *abortus-equi* is likewise due to an overflow of the second (*d*-) antigen, α phase, in the emulsion of the first (*en*-) antigen, β phase, of *amersfoort*.

Neither *abortus-equi, potsdam, brandenburg*, nor *dar-es-salaam* effected any reduction in the titre of *amersfoort* serum for the homologous second (*d*-) antigenic complex, α phase, or for the type phases of *stanley, muenchen* and *typhi*. In the same way neither *stanley, muenchen* nor *typhi* absorbed an appreciable amount of agglutinins from *amersfoort* serum for the homologous first (*en*-) antigen, β phase, or for *abortus-equi, potsdam, brandenburg* and *dar-es-salaam*.

When *potsdam* serum was absorbed by *amersfoort* all agglutinins for both *amersfoort* and *abortus-equi* were completely exhausted, but flocculation to nearly full titre was still effected with the specific phases of *potsdam, brandenburg, panama* and *london*. On reabsorbing the partly absorbed *potsdam*, serum with *panama* (factors *lv*) no appreciable agglutination resulted when specific antigens of *potsdam, brandenburg, panama* and *london* were used. *Amersfoort*, therefore, removed only the agglutinins of factors *en* from the *potsdam* serum, leaving the agglutinins of factors *lv* to the absorbed by *panama*.

The fact that *amersfoort* almost completely exhausted *muenchen* serum for the homologous specific antigen suggests that the second (*d*-) factor, α phase, is similar to the specific phase (factor *d*) of *muenchen*; the small residues of agglutinins left in *stanley* and *typhi* sera for their homologous specific antigens after absorption with *amersfoort* cannot be explained at present.

Summary and Conclusions.

A new type of pathogenic *Salmonella* for the fowl has been described. Its somatic " O " antigen corresponds with factors VI, VII of *potsdam*. It occurs only in the specific phase, but its flagellar " H " antigen contains at least two distinct and separate antigenic complexes, which commonly occur in organisms that are not even remotely related. The one complex (the first, *en*-, antigen, β phase of Kauffmann and Mitsui) contains factors *enx*, which represent also the factors of the specific phase of *abortus-equi*. The other complex (the second, *d*-, antigen, α phase of Kauffmann and Mitsui) contains factor *d*, which comprises the type phase of *stanley*, *muenchen* and *typhi*.

Single cells containing factors *enx*, on multiplying, constantly yield variants containing factor *d* as well as offspring that retain antigenic complex *enx*. In the same way single cells containing apparently only specific factor *d* will bring forth new cells, most of which retain the parental antigenic structure, but a small proportion of the progeny will acquire specific factors *enx* instead of *d*.

When a broth culture of *amersfoort* in either the *enx* or *d* phase and in an apparently pure form, is used for the preparation of sera, agglutinins of approximately the same titre for both variants are produced in the sera. The purity of the phase culture must be judged by the agglutination test, using heterologous sera which contain agglutinins either against factors *enx* or *d*.

As a result of the information given above the following antigenic structure is proposed for *Salmonella amersfoort*:

Somatic " O " antigen—VI, VII.

Flagellar " H " antigen—

(1) α phase of Kauffmann and Mitsui—*d*-

(2) β phasse of Kauffmann and Mitsui—*enx*.

II. During the course of 1937 an outbreak of a fatal disease occurred among a group of adult fowls on a farm near Onderstepoort. The symptoms and lesions presented were indistinguishable from those of an ordinary virulent outbreak of fowl typhoid. The disease was investigated by my colleague, Mr. J. D. W. A. Coles, who made spleen cultures on agar from three birds; the cultures were handed to me for identification and I spread seed material from each culture on to MacConkey's bile-salt agar in Mason tubes. After 24 hours' incubation both small and large non-lactose fermenting colonies appeared in two of the Mason tubes. Some of these colonies were picked and mixed separately with drops of a *gallinarum* serum

dilution on glass slides. All the small colonies tested were readily agglutinated by the *gallinarum* serum, but all the large colonies failed to react with this serum. A pure culture of the small colonies was obtained and labelled *culture* 360. Several of the large colonies were now tested against various " O ", type and group sera. No agglutination, whatsoever occurred with any group serum, but some of the colonies flocculated when mixed with *typhi* and *stanley* type sera, while others were agglutinated by type sera containing factors *enx, enlr, enlw* and *eh* (*abortus-equi, potsdam, dar-es-salaam, onderstepoort*). All the colonies tested were agglutinated by " O " sera containing factors VI, VII (*cholerae-suis, potsdam*). Moreover, all the colonies tested were flocculated by *amersfoort* " O " and " H " mixed sera (factors VI, VII and *d—enx*). It appeared, therefore, from these preliminary tests that the organisms from the large colonies, labelled *culture* 359, were related to *amersfoort*, and a rabbit was immunised for the production of antiserum.

When culture 359 was plated so as to give several well separated single colonies, like *amersfoort*, some of these were found to agglutinate only with a type serum containing factors *enx*, *enlv*, *enlw or eh* (*abortus-equi*, *potsdam*, *dar-es-salaam* or *reading*), while others were flocculated only by type sera containing factor *d* (*typhi*, *stanley* and *muenchen*). In order to make sure that the culture used was unquestionably pure it was single-celled. It was found that the single-cell obtained from the colony that was agglutinated by type sera containing factors *enx* etc. produced daughter organisms which, on sub-cultivation, gave rise to colonies some of which agglutinated with *enx* sera, while others (about 12 per cent.) were agglutinated by type sera containing factor *d*. Moreover, the single-cell procured from the colony that was flocculated by type sera containing factor *d* yielded bacilli, which on sub-cultivation produced colonies occurring in both the phases (*d* and *enx*). Some of the colonies (about 90 per cent.) were agglutinated only by sera containing factor *d*, while a smaller number were agglutinated by type sera containing factors *enx*, *enlr*, *enlw* or *eh*. The bacilli of culture 359, therefore, also occurred in two specific phases, the α and β phases of Kauffmann and Mitsui (1930), the organisms which occurred in the one phase constantly dissociating into bacilli which were present in both phases. As the organism occurred only in the specific phase the dissociation was confined to that phase; non-specific variants were not encountered at any time.

In order to settle the identity of culture 359, agglutination and absorption tests were carried out as shown in Table 20A. After absorption tests had been performed with *amersfoort* the identity of the strain was determined, and no further tests were performed.

The results of Table 20A show that *amersfoort* absorbed all the agglutinins (" O ", " H " *d*-type and " H " *enx* type) from the homologous serum as well as from 359 serum; on the other hand, 359 completely exhausted both its own serum and *amersfoort* serum. *Amersfoort* (culture 336) and culture 359 should, therefore, be regarded as identical. But the original *amersfoort* (culture 336), was obtained in pure culture from dead chickens during a virulent outbreak of a septicaemic disease in very young chickens at

Amersfoort, while the present strain, *culture* 359, was isolated in conjunction with *gallinarum* (*vide infra*) from adult fowls suffering from a fowl typhoid-like disease. In the epizootic discussed above (1) and in my previous paper (Henning, 1937), *amersfoort* 336 was apparently the sole cause of the mortality in the chicks; but in the present outbreak it is not quite clear whether *amersfoort* 359 or *gallinarum* 360 was the primary cause of the disease. The probability is that *gallinarum* 360 was the more important etiological agent, and that *amersfoort* 359 gained admission into the body after its resistance had been lowered by fowl typhoid.

TABLE 20A.

Antigen.	*Amersfoort* 336 Serum Absorbed by *Amersfoort.*	*Amersfoort* 336 Serum Absorbed by 359.	*Amersfoort* 336 Serum Unabsorbed.	359 Serum Absorbed by *Amersfoort* 336.	359 Serum Absorbed by 359.	359 Serum Unabsorbed.
Amersfoort "O"	0	0	800	0	0	1,600
Amersfoort "H" *d* (*a*)	0	0	12,800	0	0	12,800
Amersfoort "H" *en* (*β*)	0	0	6,400	0	0	12,800
359—"O"	0	0	800	0	0	1,600
359—"H" (*a*)	0	0	12,800	0	0	12,800
359 *en* (*β*)	0	0	6,400	0	0	12,800

0 less than 1 : 100.

Whereas the organisms comprising *amersfoort* culture 359 were motile, those of *culture* 360, obtained from the small colonies, were non-motile. *Culture* 360 was tested, therefore, both serologically and by means of fermentation reactions. The latter are given below (Table 25) and are typical for *gallinarum*. Agglutination and absorption tests were carried out with culture 360 and *gallinarum* 43, obtained from the National Collection of Type Cultures, and the serum of the latter. An antiserum for culture 360 was not prepared, but a one-sided absorption was carried out and it was found that, like *gallinarum* 43, *culture* 360, completely removed all the agglutinins from the serum of *gallinarum* 43. Moreover, the latter serum agglutinated the "O" antigen of culture 360 up to full titre (1:1600). Both serologically and by means of fermentation reactions (vide infra), therefore, culture 360 resembled *gallinarum* 43, and it should be regarded as a strain of *gallinarum*.

The fermentation reactions of *amersfoort* culture 359 are also given below (Table 25).

For purposes of comparison Table 20B is included in order to show the antigenic structure of different organisms that occur in the *α* and *β* phases of Kauffmann and Mitsui.

TABLE 20B.

Organisms known to show α-β phase variation in their specific phases (partly after Kauffmann and Tesdal, 1937).

	"O" Antigen.	H. Specific α Phase.	Antigen β Phase.	Author.	Origin.
S. Abortus-bovis	I, IV	*b*	*enx*	Bernard (1935)	Aborting cows.
S. Schleissheim	IV	*b*	*Z5*	Kauffmann & Tesdal (1937)	Bovine.
S. hvittingfoss	XVI	*b*	*enx*	Tesdal (1936)	Gastro-enteritis in man.
S. Chester	IV, V (XII)	*eh*	*enx*	Kauffman & Tesdal (1937)	Gastro-enteritis in man.
S. Brandenburg	IV (XII)	*lv*	*en*	Kauffmann & Mitsui (1930)	Gastro-enteritis in man.
S. Bispebjerg	IV (XII)	*a*	*enx*	Kauffmann (1936)a	Gastro-enteritis in man.
S. Abortis-equi	IV (XII)*	?	*enx*	—	Aborting mares.
Potsdam	VI, VII (XII)	*lv*	*en*	Kauffmann & Mitsui (1930)	Gastro-enteritis in man.
S. Oslo	VI, VII (XII)	*a*	*enx*	Tesdal (1937)	Gastro-enteritis in man.
S. Amersfoort	VI, VII (XII)	*d*	*enx*	Henning (1937)	Septicaemic disease in fowls
S. Typhi	IX (XII)	*d*	*j*	Kauffmann (1936b)	—
S. Dar-es-salaam	I, IX (XII)	lw	en	Kauffmann & Mitsui (1930)	Pyrexia.

* Kauffmann & Tesdal (1937) consider that *S. abortus-equi* will probably also be found to show α–β variation at times. This was confirmed by Edwards and Bruner (1939).

III. During spring of 1936 a very virulent epizootic occurred in a few day-old chicks at the School of Agriculture near Potchefstroom. Some of these chickens were forwarded to Onderstepoort for investigation and were examined by Mr. Coles, Chief of the Section of Poultry Diseases. Heart-blood and spleen cultures made by him yielded a pure growth of a gram-negative bacterium that was handed to me for identification. The organism was found to be very actively motile, and it did not ferment lactose. When it was tested against various " O ", type and group, sera by means of slide agglutination, it was readily agglutinated by " O " sera containing factors IV and V, by *typhi-murium* type serum and by group sera. The organism was also found to be di-phasic, and the culture was labelled 357. A rabbit was immunised with a killed saline suspension of a fresh agar culture, and a good serum was obtained. As the preliminary test indicated that culture 357 is probably related to *typhi-murium*, cross-agglutination and absorption tests were first performed with this organism (Table 20c).

TABLE 20c.

Antigen.	*Typhi-murium* (Glasgow) Serum Absorbed by *Typhi-murium.*	*Typhi-murium* (Glasgow) Serum Absorbed by 357.	*Typhi-murium* (Glasgow) Serum Unabsorbed.	357 Serum Absorbed by *Typhi-murium.*	357 Serum Absorbed by 357.	357 Serum Unabsorbed.
Typhi-murium " O ".........	0	0	800	0	0	800
Typhi-murium type..........	100	100	100,000	0	0	25,600
Typhi-murium group.........	100	100	50,000	0	0	6,400
357—" O "................	0	0	800	0	0	800
357—type..................	100	100	100,000	0	0	25,600
357—group.................	100	100	50,000	0	0	6,400

0 = 1 : 100. On account of the high titre of the *typhi-murium* type and group serum a small residue (1 : 100) of unabsorbed agglutinins were left after the absorption.

The results of Table 20c clearly show that the antigenic structures of culture 357 and *typhi-murium* (Glasgow) are identical; culture 357 removed all the agglutinins (" O ", type and group) from *typhi-murium* (Glasgow) serum, as well as from the homologous serum, while *typhi-murium* (Glasgow) completely exhausted both its own serum and 357 serum.

The agglutination (titre 1:100) which is recorded in the absorbed sera in columns 2 and 3 of Table 20c is attributed to the high titre of the unabsorbed serum; as stated above, sera of very high agglutination titres are very unwieldy for absorption tests, because it is extremely difficult to remove the last trace of agglutinin, even when the homologous antigen is used for the absorption.

After several strains of pure culture of *typhi-murium* (357) were obtained from a number of the chickens, this organism was considered to be the etiological agent of the epizootic, and an attempt was made to determine the source of the infection. As the first deaths took place only a few days after hatching, it was thought that the infection was probably obtained from the breeding hens through the eggs. Two successive slide agglutination tests were performed with the blood of the breeding stock; but both tests were negative and no carriers could be found among the hens. In the case of ducks Warrack and Dalling (1933) observed that infected eggs were laid only when the titre of the affected birds was high and that the agglutination titre of the sera obtained from reactors dropped considerably during the course of the laying season. Whether the same condition holds for fowls cannot be stated at present, and it is not certain whether the existence of carriers escaped notice on account of the lateness of the tests—serological tests were performed only some weeks after *typhi-murium* had been proved to be the cause of the epizootic. No eggs were available for examination for *typhi-murium* infection, and *Salmonellas* could not be detected in the ovaries of any of the hens examined. The source of the infection, therefore, still remains obscure.

IV. During the course of an investigation of another fowl typhoid-like epizootic among adult birds, Mr. Coles again made agar cultures from the heart-blood and spleen of the affected birds, and handed these to me for further study. The cultures (three in number) did not appear to be pure, and some seed material from each one was thinly spread on MacConkey's bile-salt agar in Mason tubes. One of the cultures yielded only lactose-fermenting colonies and was discarded; but from both the others several large and small non-lactose fermenting colonies were obtained, suggesting the existence of a mixed infection. The small colonies were readily agglutinated by *gallinarum* serum; a few of these were picked, cultured and labelled culture 415. The large colonies were tested against various "O", type and group serum dilutions on glass slides. A distinct fine granular agglutination was obtained with the "O" sera of *senftenberg* and *anatum*; coarse floccules were produced by group sera (e.g. *cholerae-suis* var. *Kunzendorf* serum) and by *newport*, *reading*, *onderstepoort* and *anatum* type sera. Some of the large colonies were sub-cultured and labelled *culture* 414, and a rabbit was immunised with it. The preliminary tests showed that *culture* 414 was related partly to *senftenberg* and *anatum* on account of its "O" antigen and partly to *newport*, *reading*, *onderstepoort* and *anatum* on account of its "H" specific antigen, and that it was di-phasic. Cross agglutination and absorption tests were, therefore, performed, first with *culture* 414, *anatum* and *senftenberg* (Table 20D).

The results of Table 20D show that culture 414 has the same antigenic structure as *anatum*. *Culture* 414 completely absorbed all the agglutinins ("O", type and group) from *anatum* serum, as well as from the homologous serum, while *anatum* completely exhausted the sera of *culture* 414 and of itself. *Anatum* var. *muenster* completely exhausted both the "O" and specific agglutinins from 414 serum, but it merely reduced the non-specific titre from 6,400 to 1,600. *Culture* 414 should, therefore, be regarded as a strain af *anatum*.

Culture 414, obtained from the small colonies, was tested against *gallinarum* 43 serum and was agglutinated by it to full titre (1:1,600); it also completely absorbed *gallinarum* 43 serum, showing that it contained the same antigenic components as *gallinarum*. The fermentation tests given below (Table 25) are also typical for *gallinarum*. *Culture* 415 should, therefore, be regarded as a strain of *gallinarum*. Antiserum for *culture* 415 was not prepared and the absorption test performed was one-sided.

In this outbreak also it is not certain whether *anatum culture* 414 or *gallinarum* 415 was the primary cause of the disease. *Salmonellas* were found in two cultures only—three were made—and both contained *anatum* as well as *gallinarum*. On account of the frequency of *gallinarum* infection, however, and on account of the predominence of *gallinarum* colonies in the first subcultures made, it seems probable that the organism of fowl typhoid was the main etiological agent in this outbreak.

In addition to these outbreaks of *Salmonella* infection in poultry, 149 epizootics of fowl typhoid and 55 outbreaks of disease in young chicks were also investigated. In most of the cases heart-blood and spleen cultures were made by Mr. J. D. W. A. Coles and submitted to me for identification. Some of the cultures were made by me personally, and *culture* 206 was obtained by Dr. Martinaglia from one of a number of chicks thought to be affected with bacillary white diarrhoea. On plating *culture* 206 I noticed that both large and small colonies appeared on the agar within 24 hours. As large and small colonies have frequently been observed in cultures of *pullorum* that were unquestionably pure, especially after allowing the cultures to stand a day or two at room temperature, the existence of a mixed infection was not suspected; nevertheless, a few of the small, and some of the large colonies were picked and cultured separately. The large colonies yielded a dense growth of actively motile organisms, while the small colonies gave rise to a much poorer growth of non-motile bacteria. In order to determine the purity of the two cultures, my colleague, Dr. J. H. Mason, kindly undertook to single-cell them; the growths obtained from the single-cells were labelled *culture* 207 and *culture* 208 for the non-motile and motile bacteria respectively.

The two cultures were now tested against various " O ", type and group sera. *Culture* 207 was agglutinated only by " O " sera containing factor IX, while *culture* 208, which proved to be monophasic, was flocculated by " H " sera containing factor *d*, as well as by " O " sera with factor IX.

With a view to carrying out complete absorption tests, antisera were prepared against both *culture* 207 and 208. But as *culture* 208 was agglutinated with sera containing " O " factor IX and " H "-specific factor *d*, it was evident that the organisms comprising the culture were closely related to *S. typhi*, which contains both these components. Absorption tests were, therefore, performed with *S. typhi*, as shown in Table 21.

The results of these tests clearly show that *culture* 208 absorbs all agglutinins (" O " and " H ") from *S. typhi* serum, as well as from its own serum, while *S. typhi* completely exhausts both the

homologous serum and 208 serum. *Culture* 208, therefore, has the same antigenic structure as *S. typhi*, and should be regarded as a strain of this organism. *S. typhi* is not regarded as a pathogen for poultry, and it is not known to be carried by fowls. It is true that Emmel (1936) claims to have isolated *S. typhi*, *S. paratyphi-A*, *S. paratyphi-B* and other *salmonellas* from the intestinal contents of fowls suffering from enteritis due to coccidiosis or verminosis, but he gives no information on what basis the organisms were recognised, and his conclusions require confirmation before they can be accepted. For comparison see biochemical tests in Table 25.

TABLE 21.

Antigen.	*Typhi* Serum Absorbed by *Typhi*.	*Typhi* Serum Absorbed by 208.	*Typhi* Serum Un-absorbed.	208 Serum Absorbed by *Typhi*.	208 Serum Absorbed by 208.	208 Serum Un-absorbed.
Typhi "O"......	0	0	3,200	0	0	1,600
Typhi "H"......	0	0	25,600	0	0	12,800
208—"O".......	0	0	3,200	0	0	1,600
208—"H".......	0	0	25,600	0	0	25,600

0 = less than 1 : 100.

As *culture* 207 was readily agglutinated by "O" sera containing factor IX, and as *pullorum* infection was suspected, absorption tests were performed with *S. pullorum*, which like *culture* 207 is non-motile. The results, which are given in Table 22, show that *culture* 207 absorbs all the "O" agglutinins from *pullorum* serum and that *pullorum* completely exhausts the serum of *culture* 207. But *pullorum* and *gallinarum* have the same somatic antigenic components, so that it is not possible to determine by means of a serological test alone to which of these two types *culture* 207 belongs; a final differentiation can be made only by means of fermentation tests (Table 25). According to these tests *culture* 207 corresponds to *Salmonella pullorum*.

TABLE 22.

Antigen.	*Pullorum* Serum Absorbed by *Pullorum*.	*Pullorum* Serum Absorbed by 207.	*Pullorum* Serum Un-absorbed.	207 Serum Absorbed by *Pullorum*.	207 Serum Absorbed by 207.	207 Serum Un-absorbed.
Pullorum "O"...	0	0	3,200	0	0	3,200
207—"O".......	0	0	3,200	0	0	3,200

0 = less than 1 : 50.
Pullorum = *Pullorum* Bb 26 of the N.C. of type cultures.

Discussion.

Of the 139 outbreaks of fowl typhoid 137 cultures were obtained from fowls and two from turkeys. Against four of these, antisera were prepared for the purpose of performing absorption tests with

strains of *gallinarum* and *pullorum* obtained from the National Collection of Type Cultures of the Lister Institute. In Table 23 the results are given which were obtained with *culture* 29; the results obtained with the other three cultures (249, 314 and 340) are similar, but are not given. Table 23 shows that *culture* 29 absorbs all the " O " agglutinins from *gallinarum* serum, as well as from the homologous serum, while *gallinarum* exhausts both its own serum and 29 serum. Similar results were obtained when *pullorum* serum was substituted for *gallinarum* serum, and *pullorum* cultures used for the absorption tests instead of *gallinarum*. *Cultures* 29, 249, 314 and 340, therefore, resemble both *gallinarum* and *pullorum* serologically, but their fermentation reactions (Table 25) corresponded to those of *gallinarum*, so that they should be regarded as strains of *gallinarum*. The other 135 cultures were used for unilateral absorption tests of *gallinarum* serum, and were found to remove all the " O " agglutinins from the serum; the fermentation reactions of all these cultures also resembled those of *gallinarum*.

TABLE 23.

Antigen.	*Gallinarum* Serum a.b. *Gallinarum*.	*Gallinarum* s. a.b. 29.	*Gallinarum* s. Unabsorbed.	29 s. a.b. *Gallinarum*.	29 s. a.b. 29.	29 s. Unabsorbed.
Gallinarum " O ".............	0	0	1,600	0	0	1,600
29—" O ".....................	0	0	1,600	0	0	1,600

Gallinarum = *gallinarum* 416 of N.C. of type cultures.
0 = less than 1 in 50.
a.b. = absorbed by.
s. = serum.

A study was also made of 55 cultures obtained from a number of few day old chicks suffering from an acute disease, and from the ovaries of hens that gave a positive agglutination test for *pullorum*. Against three of these, cultures 317, 322, and 436, antisera were prepared for absorption tests. The results, which are given in Table 24, show that *culture* 317 removes all the " O " agglutinins from *pullorum* serum and from the homologous serum, while *pullorum* also completely exhausts both these sera. Similar results were also obtained with cultures 322 and 436 and *pullorum*. When *gallinarum* was substituted for *pullorum* identical results were obtained, so that identification of the cultures could not be made entirely on the basis of the serological test—fermentation tests were necessary for the complete differentiation between *gallinarum* and *pullorum*; these are given in Table 25. Unilateral absorption tests were performed with the other 52 cultures and *pullorum* or *gallinarum* serum, resulting in the complete absorption of the sera. But when fermentation tests were carried out, it was found that the reactions of 42 of the cultures resembled those of pullorum, while the other 10 corresponded to *gallinarum*.

TABLE 24.

Antigen.	*Pullorum* Serum Absorbed by *Pullorum*.	*Pullorum* Serum Absorbed by 317.	*Pullorum* Serum Un-absorbed.	317 Serum Absorbed by *Pullorum*.	317 Serum Absorbed by 317.	317 Serum Un-absorbed.
Pullorum " O "...	0	0	3,200	0	0	1,600
317—" O ".......	0	0	3,200	0	0	1,600

Pullorum = *Pullorum* Bb. 26.
0 = less than 1 in 50.

Recently I have studied a culture of *pullorum* isolated from the spleen of a duck by a colleague, Mr. Haig.

According to the fermentation reactions, therefore, 45 of the cultures from few day old chicks and infected ovaries of adult hens should be regarded as *pullorum*, while the other 10 cultures fall under *gallinarum*. The clinical symptoms and lesions presented by the chicks from which *pullorum* cultures were isolated did not differ materially from those which yielded cultures of *gallinarum*. A diagnosis of infection with either *gallinarum* or *pullorum* in very young chicks should, therefore, not be made, unless fermentation tests have been carried out, as well as serological tests.

The fermentation reactions given in Table 25 include the rhamnose test of Bitter, Weigmann and Habs (1926), the glycerin-fuchsin-broth test of Stern (1916) and the *d*-tartrate test of Jordan and Harmon (1928). By using solid agar media advised by Jordan and Harmon more clear-cut results were obtained than with the fluid media of Silberstein (1931); in positive reactions the colour of the (phenol-red) was changed yellow by the acid formed. The the extent to which this discolouration of the agar occurred varied even with different strains of the same organism; stab cultures were made and the discolouration started from the inoculum, spreading from this point in all directions. In some cases barely a quarter of the medium was changed, while in others as much as a half or three-quarters had turned yellow.

Fifty of the strains labelled *dublin* gave negative tests with Bitter's rhamnose and Stern's glycerin-fuchsin-broth; with the d-tartrate test of Jordan and Harmon the indicator was changed yellow about half-way down the tubes (+ +) in 14 cultures, and about one quarter down the tube (+) in the remaining 36 cultures—47 of the cultures were not tested with these media. Out of 97 cultures, 76 were arabinose negative and 21 were positive after 5 days incubation; 79 were rhamnose positive and 18 were negative after 48 hours incubation. But the rhamnose was generally fermented after 4 or 5 days in the incubator. The antigenic structure of all the 97 strains was identical with that of *S. enteritidis* var. *dublin*.

TABLE 26.

Summary of results obtained with the antigenic analysis of 318 strains of Salmonellas *isolated from domestic animals and birds in South Africa.*

Type of *Salmonella*.	No. of Strains Studied.	Origin of Organisms.	"O"—Antigen.	"H"—Antigen. Type (Specific). *a*—Phase.	"H"—Antigen. Type (Specific). *β*—Phase.	"H"—Antigen. Group (non-specific).
Enteritidis* var. *dublin	97*	96 Calves and 1 human	IX, (XII)	*gp*	—	—
Enteritidis	3	Calves	IX, (XII)	*gom*	—	—
Typhi	1	Chick	IX, (XII)	*d*	(*j*?)	—
Gallinarum	149	139 Adult fowls, 10 few day old chicks	IX, (XII)	—	—	—
Pullorum	45	42 Chicks, 3 ovaries of hens	IX, (XII)	—	—	—
Abortus-equi	1†	Adult horse (tendo-vaginitis)	IV, (XII)	—	(*enx*.)	—
Typhi-murium	10	2 Calves, 2 canaries, 1 finches, 1 canary-food, 1 pig, 1 chickens, 1 sheep, 1 rabbits	IV, V (XII)	*i*	—	1, 2, 3.
***Typhi-murium* var. *Copenhagen* (*Storrs*)**	1	Foal (purulent arthritis)	IV, (XII)	*i*	—	1, 2, 3.
Cholerae-suis (Kunzendorf)	6	Pigs	VI, VII (XII)	—	—	1, 3, 4, 5.
Bovis-morbificans	1	Pork	VI, VIII	*r*	—	1, 3, 4, 5.
Anatum	1	Fowls	X, III	*eh*	—	1, 4, 6.
Onderstepoort	1	Sheep	XIV	*eh*	—	1, 4, 5.
Amersfoort	2	1 Chickens, 1 adult fowls	VI, VII, (XII)	*d*	*enx*	—

*** Two of these strains were non-motile and were, therefore, devoid of H-specific factors *gp*.**

† The one strain studied had lost its motility and, therefore, did not contain an H—antigen, factors *enx*.

O—factor (XII) has been recently added by Kauffmann (1935b) and is supposed to occur in a number of different species that are not related.

Since the completion of this paper 10 more strains of *dublin* have been isolated from calves and 17 more strains of *gallinarum* have been obtained from outbreaks of fowl typhoid. In their antigenic structure and biochemical reactions the *dublin* strains correspond to those *dublin* strains described above and the reactions of the *gallinarum* strains resembled those of the *gallinarum* cultures given above. The total number of *dublin* strains therefore, should be 107 and of *gallinarum* 166, and the total number of *Salmonellas* 345.

For comparison five stock strains of *dublin* were studied. Of these Pesch 256, Cambridge 1 and Topley were positive with Stern's glycerin-fuschin-broth, while *dublin* (Knox) and *Paracoli* (Savage) 255 gave a negative reaction. All five reacted negatively with Bitter's rhamnose and positively with Jordan and Harmon's d-tartrate. On comparing the antigenic structure of Cambridge 1 and Topley with that of *dublin* (Knox) by means of agglutination and absorption tests the three cultures were found to be identical; both Cambridge 1 and Topley completely exhausted *dublin* (Knox) serum.

Of the three strains which corresponded antigenically to *S. enteritidis* all gave a positive reaction to Bitter's rhamnose, two (cultures 290 and 418) reacted positively with Stern's fuchsin-broth and one (culture 216) failed to change it. All three cultures were positive with Jordan and Harmon's medium. The three stock strains, M.7. and D.5, and Weybridge gave a positive reaction with Bitter's rhamnose, Stern's fuchsin-broth and Jordan and Harmon's d-tartrate.

Of the 13 strains which were antigenically identical with *typhi-murium* (Glasgow) all were Stern and d-tartrate positive, twelve were Bitter positive and one (culture 357) was Bitter negative. Eight of the cultures were inosite positive and two (strains 357 and 502) were negative; nine were positive and one (strain 357) was negative with rhamnose, while all the ten tested fermented arabinose. Culture 357 was both rhamnose and inosite negative. Moreover, a complete mirror absorption test performed with culture 357 (Table 20c) showed without doubt that it is a strain of *typhi-murium*. Of the *typhi-murium* stock strains tested all five were Bitter and d-tartrate positive, three (Mutton 74, Glasgow and Weybridge) were Stern positive, while two (Binns and Breslau) were Stern negative.

The one strain of *typhi-murium* var. *Copenhagen-Storrs*-(culture 478) studied gave positive Bitter, Stern and d-tartrate reactions. It fermented both inosite and maltose, but not arabinose; while the stock strain *Storrs* 19500 fermented arabinose and inosite, but not maltose; it was also Bitter and d-tartrate positive, but Stern negative. The two strains of *Copenhagen* (659 and 1147) both fermented maltose and arabinose but not inosite; both were Bitter, Stern and d-tartrate positive. Antigenically, however, *Copenhagen* (*Storrs*) 478, *Storrs* 19500 and the two strains of *Copenhagen* were identical (Table 12).

For comparison 5 stock strains of *paratyphi-B* were included in the test. Of these three (D.C., Grey and Odense) were Stern positive and two (Schottmuller and Lowestoft) were negative; only one (Grey) was Bitter positive, the other four were negative. All five reacted negatively with Jordan and Harmon's d-tartrate.

The six strains which resembled *cholerae-suis* (Kunzendorf) antigenically were all Bitter and d-tartrate positive but Stern negative.

There was only one culture (strain 391) which had the same antigenic structure as *bovis-morbificans*. Like the original culture

of Basenau it was Bitter, Stern and d-tartrate positive, but the strain of Sladden and Scott differed from it on account of its negative Bitter reaction.

The strain of *anatum* (culture 414) studied gave a positive reaction with Bitter, Stern and d-tartrate, resembling, therefore, the two stock strains (3701 and 3702).

Like *abortus-equi* W.H.2, the one strain (culture 219) studied reacted positively to Bitter and d-tartrate, but negatively to Stern's glycerin-fuchsin-broth. It was found to be anaerogenic.

Both strains of *amersfoort* and the one of *onderstepoort* were positive with Stern, Bitter and d-tartrate.

All the forty *gallinarum* strains tested gave a negative reaction with Bitter and Stern, but a positive one with Jordan and Harmon's d-tartrate. Out of 139 strains 119 fermented arabinose, while 20 failed to do so after 4 days. All the cultures fermented rhamnose, but in the case of 17 of the strains the fermentation was delayed. All were dulcite and maltose positive.

The fifteen strains of *pullorum* tested all failed to react on Bitter, Stern or d-tartrate. Thirty-seven of the 45 strains fermented arabinose, but 8 failed to do so. All the 45 strains fermented rhamnose, but in 10 the reaction was delayed. All were dulcite and maltose negative, and all 45 strains were anaerogenic. (Recently a few aerogenic strains were isolated.)

For the differentiation of *S. gallinarum* and *S. pullorum* the cysteine-gelatin medium of Hinshaw and Rettger (1936) was tried, but the organisms failed to grow in the medium so that no change could be detected in the tubes. The cysteine used was freshly prepared.

Only one strain of *S. typhi* was studied. The original culture, obtained from a chicken by Martinaglia, was found to be composed of a mixture of two organisms when I received it; the one non-motile and the other motile. After "single-celling", the non-motile culture was labelled "strain 207" and the motile one "strain 208". The former resembled *pullorum* and *gallinarum* antigenically (Table 22) and *pullorum* biochemically; while culture 208 was found to have the same antigenic structure as *typhi* (Table 21). The fermentation reactions of the latter, however, did not altogether correspond to those of the stock strains of *typhi* employed. Like *typhi*, culture 208 was Bitter and Stern negative, and d-tartrate positive. Four years ago culture 208 fermented dulcite slowly, and maltose within 24 hours, but at present it fails to ferment dulcite and the fermentation of maltose is delayed for about five days. All the stock strains of *typhi* used fermented maltose within 24 hours, but failed to ferment dulcite. The fermentation reactions of culture 208 are, therefore, not quite typical of *typhi*, but its antigenic structure is identical with that of this bacterium. When it was found that a change had occurred in the fermentation reactions of culture 208 after four years, the serological tests were repeated. The results of these tests show that the antigenic structure of culture 208 has remained unaltered, and that it still resembles *typhi*.

When the biochemical reactions of the different types of *Salmonellas* studied are compared with their serological reactions very marked differences may be exhibited by several strains belonging to the same serological type. For example, some striking variations in their fermentation reactions were manifested by the four strains of *typhi-murium var. storrs* (*Copenhagen*) studied; the one strain 478 described by me and the other 3 stock strains. All four gave positive Bitter and d-tartrate tests; three (*Storrs* 478 and *Copenhagen* 659 and 1147) were Stern positive and fermented maltose, while one (*Storrs* 19500) gave a negative reaction to these tests. Two (*Storrs* 478 and 19500) were inosite positive and two (*Copenhagen* 659 and 1147) were negative. All four were antigenically identical (Table 12).

Analogous variations have been observed in the case of *typhi-murium*, *paratyphi-B*, *enteritidis*, *enteritidis* var. *dublin*, *bovis-morbificans* and, to a lesser degree with *typhi*, *pullorum* and *gallinarum*.

SUMMARY.

Altogether 318 strains of *Salmonella* from different species of animals have been studied. The antigenic analysis obtained is summarised in Table 26 and the biochemical reactions are given in Table 25.

The results recorded in Tables 25 and 26 therefore, show that the biochemical reactions of *Salmonellas*, although very useful as complementary tests, cannot be solely relied upon for the identification of the type or species of organism. Many of the types which were indistinguishable on serological grounds were found to differ in their fermentation reactions, whereas others which differed markedly in their antigenic composition showed identical biochemical reactions. For the recognition of the type of *Salmonella* studied, reliance can be placed only on the antigenic analysis of the bacterium by means of carefully performed serological tests.

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REFERENCES.

ANDREWES, F. W. (1922). Studies in group agglutination. 1. The Salmonella group and its antigenic structure. *Jl. Path. and Bact.*, Vol. 25, pp. 505-521.

ANDREWES, F. W. (1925). Studies in group agglutination. 11. The absorption of agglutinins in diphasic salmonellas. Ibid., Vol. 28, pp. 345-359.

ANDREWES F. .W. and NEAVE (1921). The nature and systematic position of *B. paratyphosus C. Br. Jl. Exp. Path.*, Vol. 11, pp. 157-174.

ARKWRIGHT, J. A. (1921). Variation of bacteria in relation to agglutination both by salts and by specific sera. *Jl. Path. and Bact.* Vol. 24, pp. 36-60.

ARKWRIGHT, J. A. (1931). Agglutination. A system of *Bact. M.R.C.*, Vol. 6, pp. 381-423.

ARNBERGER, A. (1931). Ueber das Vorkommen von Paratyphus-enteritis-Bakterien bei Darmerkrankungen der Pferde. *Tierartzl. Rundschau*, Vol. 37, pp. 689-692.

BAARS, G. (1929). Ueber eine Fleischvergiftung nach Genuss von geräucherter Gänsebrust mit Betrachtungen über die Notwendigkeit der Herstellung Stütlich geprüfter, hochwertiger, typen spezifischer agglutinierender Sera. *D.T.W.*, Vol. 37, No. 30, pp. 467-471.

BAARS, G. (1931). Fleischvergiftung nach Genuss geraucherter Gänsebrust. *Z. Fleisch. u. Milchhyg.*, Vol. 41, pp. 521-523.

BAINBRIDGE, F. A. (1908). On the paratyphoid and foodpoisoning bacilli, and on the nature and efficiency of certain rat viruses. *Jl. Path. and Bact.*, Vol. 13, pp. 443-466.

BAINBRIDGE, F. A. (1911). The aetiology and epidemiology of paratyphoid fever and "food-poisoning". *Proc. Roy. Soc. Med.*, Vol. 4. No. 2, Ebid., 51-72.

BAINBRIDGE. F. A. and O'BRIEN, R. A. (1912). The paratyphoid group of bacilli. *Jl. Hyg.*, Vol. 11, pp. 68-88.

BASENAU, F. (1894). Ueber eine Fleisch gefundene infectiöse Bakterie. *Arch. f. Hyg.*, Vol. 20, pp. 242. Quoted by Sladden and Scott.

BAUER, J. T. and MCLINTOCK, M. (1929). Fatal Septicaemia in man due to Bacillus (Salmonella) Suipestifer. *Jl. Inf. Dis.*, Vol. 44, pp. 292.

BAUMANN, R. and GRATZL, E. (1931). Paratyphus-Breslau-Erkrankungen beim Pferd. Wien *Tierartzl. W. Sch.*, Vol. 18, pp. 322-336, 353-359.

BEAUDETTE, F. R. (1926a). *B. aertrycke* infection in canary birds and parrots. *Jl. A.V.M. Ass.*, Vol. 21, pp. 642-643.

BEAUDETTE, F. R. (1926b). *B. aertrycke* as an etiological agent in a disease affecting Squabs. Ibid., Vol. 21, pp. 644.

BEAUDETTE, F. R., AND EDWARDS, P. R. (1926). The Etiology of Canary Bird Epizootic. *Jl. Bact.*, Vol. 12, pp. 51-55.

BECK, A., UND MEYER, E. (1927). Enzootische Erkrankungen der Tauben durch Bakterien der Paratyphus-Enteritidis-Gruppe. *Z. Inf. Haust.*, Vol. 30, pp. 15-41.

BECK, A. (1929). Beitrag zum Verhalten der Tauben-paratyphusstämme. *Z. f. Inf. Haust.*, Vol. 35, pp. 124.

BELLER, K. (1933). Die Bedeutung des Geflügels für die Entstehung von Lebensmittelvergiftungen. *Z. Fleisch. und Milchhyg.*, Vol. 43, pp. 365-369.

BERGE, R. (1929). Enteritiserkrankung bei Tauben (*B. enteritidis breslaviense*), D.T.W., Vol. 37, pp. 247-249.

BERNARD, H. (1935). Ein neuer Bakterientyp aus der Paratyphus-Enteritidis-Gruppe als Erreger des Verkalbens in einen Rinderbestande. *Z. Hyg.*, Vol. 117, pp. 352-357.

BEYER, H. G., AND REAGH, A. L. (1904). Further differentiation of Flagellar and Somatic Agglutinin. *Jl. Med. Res.*, Vol. 12, p. 313.

BIEN, Z., UND SONNTAG, F. (1917). Herstellung eines haltbaren Fleckfieber diagnostikums. *Munch. Med. Wochschr*, Vol. 64, p. 1409.

BIEN, Z. (1924). Ueber das Alkohol-Fleckfieber-diagnostikum. *Z. bl. Bakt.*, Vol. 93, pp. 196-200.

BITTER, L., WEIGMANN, F. UND HABS (1926). Bestimmung der gebildeten Saüermenge zur Unterscheidung verwandter Bakterien. Rhamnosereaktion zur Differentierung von Paratyphus B und Breslaubakterien. *Münch. Med. Woch.*, Vol. 73, pp. 940-941.

BORDET, J. (1899). Mécanisme de L'Agglutination. *Annal. Inst. Pasteur*, Vol. 13, pp. 225-250.

BORNSTEDT VON, S., UND FIEDLER (1932). Untersuchungen auf Fleischvergiften bei aus Polen und Litauen Angeführten Mastgansen. *B.T.W.*, Vol. 48, pp. 721-724.

BOSWORTH, T. J. (1933). The Diagnosis of *Bacterium abortus ovis* Infection by Agglutination. *Third Rep. Inst. An. Path.*, Univ. of Cambridge (1932-33), pp. 19-32.

BOSWORTH, T. J., AND GLOVER, R. E. (1925). Contagious Abortion in Ewes. *Vet. Jl.*, Vol. 81, pp. 319-334.

BOSWORTH, T. J., AND LOVELL, R. (1931). The Dublin type of Salmonella as a cause of disease in Calves. *Sec. Rep. Inst. Animal Path.*, Univ. of Cambridge, pp. 21-27.

BOYCOTT, A. E. (1906). Observations on the Bacteriology of Paratyphoid Fever, and on the reactions of typhoid and Paratyphoid Sera. *Jl. Hyg. Camb.*, Vol. 6, pp. 33-73.

BOYCOTT, J., AND McNEE, J.W. (1936). Human infection with the American Hog Cholera Bacillus. *Lancet*, Vol. 231, pp. 741-742.

BOURMER UND DOETSCH (1928). Ueber eine durch den *Bacillus enteritidis* Gaertner hervorgerufene Seuchenhafte Erkrankung in den Rinderbestande des Gutes Kärthausenhof bei Koblenz und eine durch Käse verursachte Uebertraging auf den Menschen. *Z. Fl. u. Milchhyg.*, Vol. 38, pp 389-391.

BRANHAM, S. E., MOTYCA, L. J., AND DEVINE, C. J. (1930). Bacteremia due to *Salmonella Suipestifer*. *J.A.M.A.*, Vol. 94, p. 1758.

BRAUN, H (1918). Das Wesen der Weil-Felix'sche Reaktion auf Fleckfieber. *Berl. Klin. Wsch.*, Vol. 55, p. 637.

BRAUN, H., UND SCHAEFFER, H. (1919). Zur Biologie der Fleckfieber-Proteusbazillen. *Z. Hyg.*, Vol. 89, pp. 339-362.

BRIDGES, R. F., AND SCOTT, W. M. (1935). *J. Roy. Army Med. Corps*, Vol. 65, pp. 221-223.

BROWN, M. H. (1936). Criteria for the selection of suitable strains of *B. typhosus* for use in the preparation of Typhoid Vaccine. *Canad. Publ. Hl. Jl.*, Vol. 27, No. 4, pp. 171-175.

BRUNER, D. W. and EDWARDS, P. R. (1939). A note on the monophasic non-specific Salmonella types. *Jl. Bact.*, Vol. 37, pp. 365-370.

BRUNER, D. W. and EDWARDS, P. R. (1939). Micro-organisms of the *Salmonella choleroe-suis* group isolated in the United States. *Am. Jl. Hyg.*, Vol.. 30, pp. 75-81.

BRUNS UND GASTERS (1920). Paratyphusepidemie in einer Hammelherde: dadurch bedingte Massenerkrankungen an Fleischvergifting in Überruhr. *Z. Hyg.*, Vol. 90, pp. 263-280.

BRUNS UND FROMME (1934). Ueber Nahrungsmittel Erkrankungen durch Enteneier. *Münch. Med. W.Sch.*, Vol. 81, pp. 1350-1352.

BRUNETT, E. L. (1930). Paratyphoid Infection of Pigeons. *Cornell Vet.*, Vol. 20, pp. 169-174.

BURGHOFFER, G. (1927). Beitrag zum Paratyphus des Wassergeflügels. *Allat. Lapok.*, Vol. 50, pp. 232-233.
E.S. Jahresbericht, Vol. 47, No. 2, p. 1321 (1927).

CASH, J. R., AND DOAN, C. A. (1931). Spontaneous and Experimental Infection of Pigeons with *B. aertrycke*. *Am. Jl. Path.*, Vol. 7, pp. 373-397.

CASTELLANI, A. (1902). Die Agglutination bei gemischter Infection und die Diagnose der Letzteren. *Z. Hyg. Infkr.*, Vol. 40, pp. 1-19.

CERNAIANU, C., AND POPOVICI, I. (1933). Sur L'Infection Paratyphique Spontanée du Pigeon par les Bacilles du Type Aertryck du Breslau. *Compt. Ren. Soc. de Biol.*, Vol. 112, pp. 829-832.

CERNOZUBOV, N., PILIPOVIE, D., UND STAVEL, J. (1937). Zur Salmonellafrage. Serobakteriologische, epidemiologische und Klinische Untersuchungen das Jahre 1931-1934. *Z. bl. Bakt.* I (orig.), Vol. 138, pp. 460-483. Extr. *Vet. Bull.*, Vol. 8, No. 3, p. 135.

CHERRINGTON, V.A., GILDOW, E. M., AND MOORE, P. (1937). Paratyphoid in Turkeys. *Poultry Sc.*, Vol. 16, No. 4, pp. 226-231.

CHRISTIANSEN, M. (1915). Paracolibacillose hos Kraeget Saertryk of Maanedskrift for Dyrlaeger, Vol. 24 (quoted by Jensen).

CHRISTIANSEN, M. (1914). Ueber das Vorkomen von Nicht-gasproduzierende Paracolibacillen in Fällen von Paracolibacillose beim Kalbe. *Z. bl. f. Bakt.*, Vol. 74, pp. 474-481.

CLARENBURG, A. (1931). Voedselvergiftingen door Bakterien uit de Salmonellagroep. *Tydsch. Diergen.* Vol. 58, pp. 1098-1111. Abstr. *Vet. Bull.*, Vol. 2, pp. 596-597.

CLARENBURG, A. (1933). Bacteriologische en Serologische Onderzoekingen in verband met paratyphus by het Kalf. *Tydsch. v. Diergen*, Vol. 60, No. 20, pp. 1092-1100.

CLARENBURG, A. (1934). Bakteriologische Vleesonderzoek by Paratyphose van den Rund. De beoordeeling van zogenoemde lokale Parahyphus uit de oogpunt van Vleeskeuring. Ibid., Vol. 61, No. 17, pp. 907-919.

CLARENBURG, A., EN DORNICKX, C. G. J. (1932). Nahrungsmittelvergiftung bei Menschen in Zusamenhang mit Taubenparatyphose. *Z. Hyg.*, Vol. 114, pp. 31-41.

CLARENBURG, A., EN POT, A. W. (1935). Voedselvergiftiging by den Mensch in verband met Eendenparatyphose *Tydsch. v. Diergen*, Vol. 62, pp. 1240-1246.

CLAYTON, E. S., MILNE, V. E., AND MENTON, J. (1930). Outbreak of food poisoning in Staffordshire. *B.M.J.*, 1930, No. 2, p. 685.

COMBES, R. (1911). Etude Bacteriologigue des Affections Typhoides sur des chevaux atteints a Lyon, Nantes et Saint-Nazaire. *Compt. Rend. Soc. de Biol.*, Vol. 70, pp. 73-74.

COOPER, G. M., AND KRUMWIEDE, C. (1924). A note on the serological characters of *Bact. Anatum* (Rettger). *Abst. Bact.*, Vol. 8, p. 25.

CRAIGIE, J. (1931). Studies on the serological reactions of the Flagella of *B. Typhosus*. *J. Immunology*, Vol. 21, pp. 417-511.

CRAIGIE, J., AND BRANDON, K. F. (1936). The Laboratory Identification of V-forms of *B. typhosus*. *Canad. Publ. Hl. Jl.*, Vol. 27, pp. 165-170.

CRAIGIE, J., AND BRANDON, K. F. (1936). Bacteriophage spesific for the "O" Resistant V-forms of *B. typhosus*. *Jl. Path. and Bact.*, Vol. 48, pp. 233-248.

DALLING, T., AND WARRACK, G. H. (1932). Ducks and Salmonella Infection. *Jl. Path. and Bact.*, Vol. 35, p. 655.

DAMMANN UND STEDEFEDER (1910). Untersuchungen über Scheinepest. *Arch. f. Wiss. Tierheilk.*, Vol. 36, pp. 432-484.

DASSONVILLE, C., AND RIVIERE, C. (1913). Contribution á l'etude de l'avortement epizootique des Juments. *Rev. Gén. Med. Vet.*, Vol. 21, pp. 237-252.

DAUBNEY, R. (1927). Paratyphoid infection of calves in Kenya. *Vet. Rec.*, Vol. 7, pp. 793-802.

DE JONG, D. A. (1913). Ueber einen Bacillus der Paratyphus-B.-Enteritis-Gruppe als Ursache einer Seuchenhaften Abortus der Stute. *Z. bl. Bakt.* I, Orig., Vol. 67, pp. 148-157.

DE SCHWEINITZ, E. A., AND DORSET, M. (1904). New Facts concerning the Etiology of Hog Cholera. *20th Rep. Bureau of Animal Industry. Dept. of Agric., U.S.A.*, pp. 157-162.

D. HOOGHE (1932). Essai sur l'Etude de le Fievre Paratyphoide C. *Ann. Soc. Belg. Trop.*, Vol. 11, pp. 168-182.

DIENES, L. und WAGNER, R. (1918). Ueber Paratyphus-B Infektionen. *Z. f. Hyg.*, Vol 87, p. 157.

DITTHORN, F. (1913). Ueber das Verhalten der Typhus und Typhus ähnlichen Bacillen (Paratyphus A, B und Enteritis Gaertner) zu Verschiedenen Zuckerarten und diesen nahestehenden neher mehratomigen Alkoholen. *Z. bl. Bact.*, Vol. 67, pp. 497-509

DODD (1905). Epizootic Pneumo-enteritis in the Turkey. *Jl. Comp. Path. and Ther.*, Vol. 18, pp. 239-245.

DORSET. M., BOLTON, B.M. and McBRYDE, C. N. (1905). The etiology of hog cholera. *21st Rep. Bureau of Animal Industry., Dept. of Agric., U.S.A.* 138-158 and Bull. 72 of 1905.

DOUMA, S. (1916). Het voorkomen van enteritis ot Paratyphus-B bacillen by Kalveren. *Tydsch. v. Diergen.*, Vol. 43, pp. 720-741.

DOYLE, T. M. (1927). *B. aertrycke* infection in chicks. *J. Comp. Path. and Ther.*, Vol. 40, pp. 71-75.

DREYER. M. (1909). Widal's Reaction with Sterilised Cultures. *Jl. Path. and Bact.*, Vol. 13, pp. 331-337.

DUNNING, F. J. (1934). Observations on Kiel Disease in Ducklings occurring in South Africa. *Vet. Rec.*, Vol. 14, pp. 423-424.

EDWARDS, P. R. (1929). A fatal infection of chicks due to bacilli of the paratyphoid-B. Group. *Jl. Inf. Dis.*, Vol .45. pp. 191-195.

EDWARDS, P. R. (1934). *Salmonella Aertrycke* in Colitis of Foals. *Jl. Inf. Dis.*, Vol. 54, pp. 85-90.

EDWARDS, P. R. (1935a). The Antigens of *Salmonella anatum*. *J. Bact.*, Vol. 30, pp. 269-275.

EDWARDS, P. R. (1935b). A Serological Variant of *S. aertrycke* isolated from Pigeons. *Jl. Bact.*, Vol. 30, pp. 465-471.

EDWARDS, P. R. (1936). The Occurrence of Salmonella, Oranienburg Type in an Infection in Quail. *Jl. Bact.*, Vol. 32 pp. 259-263.

EDWARDS, P. R. (1937a). The occurrence of Salmonella, Seftenberg type, in a disease of turkeys. *Jl. Bact.*, Vol. 33, No. 2, pp. 193-195.

EDWARDS, P. R. (1937b). Paratyphoid infection in fowls. *J.A.V.M.Ass.*, Vol. 43, No. 3, p. 403.

EDWARDS, P. R. (1938). Further studies on IV-varients of *Salmonella typhimurium* (aertrycke) with special reference to cultures from pigeons. *Jl. Bact.*, Vol. 35, No. 2, pp. 123-127.

EDWARDS, P. R. and BRUNER. D. W. (1939). The demonstration of phase Variation in *Salmonella abortus-equi*. *Jl. Bact.*, Vol. 38, pp. 63-72.

EDWARDS, P. R., and RETTGER, L. F. (1924). Relation of *Bact. anatum* to *Bact. paratyphosus B* and *Bact. suipestifer*. Abst. *Bact.*, Vol. 8, p. 25.

EDWARDS, P. R., and RETTGER, L. F. (1926). The partyphoid B-suipestifer group of Bacteria. *Jl. Bact.*, Vol. 13, pp. 73-97.

EISENBERG, P., und VOLK, R. (1902). Untersuchungen über die Agglutination. *Z. Hyg. Infekt. Kr.*, Vol. 40, pp. 155-195.

ELKELES, G. (1925). Fleischvergiftungen in Deutschen Reich in Jahre 1923. *Z. Fleisch u. Milchhyg.*, Vol. 35, pp. 12-13.

ELKELES, G., und STANDFUSS, R. (1931). Die paratyphosen. *Handbuch der Pathogenen Mikro-organismen* (Kolle Kraus und Uhlenhuth) Bd. III, No. 2, pp. 1585-1826.

EMMEL, M. W. (1929). Arthritis in Pigeons caused by *Salmonella Schottmulleri*. *Jl. A.V.M. Ass.*, Vol. 28, pp. 367-370.

EMMEL, M. W. (1936) .The Etiology of Fowl Paralysis Leukemia and allied conditions in animals. III. The Intestinal Flora of Chickens affected with Enteritis associated with Intestinal Parasitism *Florida, Agr. Exp. Sta.*, Bull. 293.

FELIX, A., and PITT, R. M. (1934). Virulence of *B. typhosus* and Resistance to "O" antibody. *Jl. Path. and Bact.*, Vol. 38, pp. 409-420.

FELIX, A., and PITT, R. M. (1934). A new antigen of *B. typhosus*. *Lancet* Jul. No. ii, p. 186.

FELIX, A. and PITT, R. M. (1935). The virulence of immunogenic activities of *B. typhosus* in relation to its antigenic constituents. *Jl. Hyg.*, Vol. 35, pp. 428-436.

FELIX, A., and PITT, R. M. (1936). The Vi antigen of various Salmonella types. *Brit. Jl. Exp. Path.*, *Vol.* 17, *pp.* 81-86.

FELIX, A., and BHATNAGAR, S. S. (1935). Further observations on the proteries of the Vi-antigen of *B typhosus* and its corresponding antibody. *Br. Jl. Exp. Path.*, Vol. 16, pp 422-433.

FELIX, A., BHATNAGAR, S. S., and PITT, R. M. (1934). Observations on the properties of the Vi-antigen of *B. typhosus*. *Br. Jl. Exp. Path.*, Vol. 15, pp. 346-354.

FELIX, A., und MITZENMACHER, F. (1918). *Wien. Klin. W.Schr.*, Vol. 31, p. 988. (Quoted by Arkwright, 1931.)

FELIX, A., and OLITSKI, L. (1925). Serumbactericidie und qualitative Rezeptoren-Analyse. *Klin. W. Sch.*, Vol. 4, p. 72.

FELIX, A., and OLITSKY, L. (1928). The use of preserved bacterial suspensions for the agglutination test, with special reference to the enteric fevers and typhus fevers. *Jl. Hyg.*, Vol. 28, p. 55-66.

FICKER, M. (1903). *Berl. Klin W. Schr.*, Vol. 40, p. 1021. (Quoted by Arkwright, 1931.)

FRANCK (1876). Handbuch d. Tierärztl. Geburtsh. (cited by C. O. Jensen).

FRANCKE, G., STANDFUSS, R., und WILKEN, H. (1934). Die Bekampfung der Enteritidiserkrankung des Rindes nebst weiteren Beobachtungen über diese Krankheit. *Berl, T. W.*, Vol. 50, pp. 753-759. *Vet. Jnl.*, Vol. No. 10, pp. 737-738.

FRICKINGER, H. (1919). Fleischvergiftungsepidemie in Anschluss an eine Paratyphuserkrankung beim Schaf. *Z. Fleisch. u. Milchhyg.*, Vol. 29, pp. 346-357.

FROMME, W. (1933). Zur Versuche von Nahrungsmittelvergiftungen durch Enteneier. *D. Med. Wochsch.*, Vol. 59 ,No. 17, p. 28, April.

FUJIMURA, S. and HOSHI, T. (1936). A case of infection with *Bact, abortus-equi in man. Jl. Jap. Soc. Vet. Sc.*, Vol. 15, pp. 159-163, No. 1. Engl. Summary pp. 28-29, No 2.

FURTH, E., und KLEIN, K. (1933). Neue Feststellungen über Entstehungsursache von Enteritidis-Gruppenerkrankungen (Enteritis und Enterei). Veröff a.d. Geb. d. *Medizinalverwaltung*, Vol. 39, H 3 Berlin. Quoted by Meyer.

GAIGER, S. H. and DAVIES, G. O. (1930). "Keel Disease" in Ducklings in Britain. *Jl. Comp. Path. and Ther.*, Vol. 43, pp. 125-141.

GARROW, R. P. (1920). A case of paratyphoid C fever in East Africa. *Lancet*, Vol. 1, p. 1221.

GIGLIOLI, G. (1930). Paratyphoid C—and endemic disease in British Guiana. *Jl. Hyg.*, Vol. 29, No. 3, pp. 273-281.

GILRUTH, J. A. (1910). Diseases in Canaries. *Vet. Jl.*, Vol. 17, pp. 655-658.

GLAGE (1916). Aus der Gerichtspraxis bei Fleischvergiftungen. *B.T.W.*, Vol. 32, pp. 517-523, pp. 532-534.

GLASSER, K. (1909). Zum heutigen stande der Schweinepestfrage und zu den weiteren Untersuchungen Uhlenhuth, Hübener Xylander und Bohtz über das Wesen und Bekämpfung der Schweinepest und über die Bakteriologie der Hogcholera (Paratyphus B)-gruppe. *D.T.W.*, Vol. 17, pp. 513-516 und pp. 5291534.

GOOD, E. S., AND CORBETT (1913). Investigations of the Etiology of Infectious Abortion of Mares and Jonnets in Kentucky. *Jl. Inf. Dis.*, Vol. 13, pp. 53-68.

GOOD, E. S., AND SMITH, W. V. (1914). The *Bacillus Abortivus equus* as an Etiological Factor in Infectious Arthritis in Colts. *Jl. Inf. Dis.*, Vol. 15, pp. 347-349.

GRAHAM, R. (1936). Salmonella isolated from baby Quail. *Jl. A.V.M.Ass.*, Vol. 41, pp. 763-764.

GRAHAM, R., REYNOLDS, F. H. K., AND HILL, J. F. (1919). Bacteriological Studies of a Peracute Disease of Horses and Mules. *Jl. Am. Vet. Med. Ass..*, Vol. 56, pp. 378-393, pp. 489-507, pp. 586-599.

GREENFIELD, E. C., AND JUDD, M. H. (1936). *Salmonella suipestifer var. Afri. Aust.* in an outbreak of Food-poisoning in the Cape Peninsula. *S.A. Med. Jl.*, Vol. 10, No. 15, pp. 544-545.

GRUSCHKA, T. (1920). Serologische Beziehungen Zwischen *B. typhi abdominalis, B. enteritidis Gaertner*, und *B. typhi gallinarum. Z. Imm. Forsch.*, Vol. 30, pp. 208-226.

GRUSCHKA, T. (1923). Variationsversuche mit dem *B. enteritidis Gärtner. Ibid*, Vol. 35, p. 37.

HARKINS, M. J. (1926). *Bacillus Paratyphosus B* Infection of Canary Birds. *Jl. A.V.M.Ass.*, Vol. 22, No. 3, pp. 376-378.

HEIMANN, W. (1912). Ueber die durch einen sogenannten Paratyphus-C Bacillus verursachte Fleischvergiftungs-epidemie in Hildesheim im Früjahr 1911. *Zbl. f. Bakt.* (1 Abt. orig.), Vol. 66, pp. 211-221.

HENNING, M. W. (1932). Paratyphoid Disease in Calves. *Animal Diseases in S.A.*, Vol. 1, pp. 88-93.

HENNING, M. W. (1936). *Salmonella Onderstepoort:* A new type of Salmonella from a sheep. *Jl. Hyg.*, Vol. 36, No. 4, pp. 525-531.

HENNING, M. W. (1937). On the Variation of the Specific Phase of *Salmonella Amersfoort* N. Sp. *Jl. Hyg.*, Vol. 37, pp. 561-570.

HENNING, M. W. (1938). Calf's Meat as a cause of Paratyphoid in Natives *S.A. Med. Jl.*, Vol. 12, No. 2, pp. 53-55.

HENNING, M. W., AND CLARK, R. (1938). Purulent Arthritis in Foals due to *Salmonella typhi-murium*, var. Copenhagen. *Jl. S.A.V.M.Ass.*, Vol. 9, pp. 179-182.

HENNING, M. W., AND HAIG, D. A. (1939). Serological Variants of *S. typhi-murium* isolated from South African Animals. *Onderstepoort Journal of Veterinary Science* (in the press).

HENNING, M. W., AND GREENFIELD, E. C. (1937). *Salmonella bovis morbificans* (Basenau) from an outbreak of food poisoning in the Cape Province. *Onderstepoort Jl. of Vet. Sc.*, Vol. 8, No. 2, pp. 351-355.

HENNING, O. (1894). Rapport over Leverziekte onder Kalveren. Dept. van Landbou, Kaap de Goede Hoop.

HENNING, O. (1894). Liver disease in calves. *Agr. Jl., Cape of Good Hope*, Vol. 7, pp. 310 and 327.

HINSHAW, W. R., AND RETTGER, L. F. (1936). Cysteine gelatin as a differential medium for *S. pullorum* and *S. gallinarum*. *Proc. Soc. Exp. Biol. and Med.*, Vol. 35, No. 1, pp. 44-46.

HIRSCHFELD, L. (1919). A new germ of paratyphoid. *Lancet.*, 1919, No. 1, p. 296.

HOFFMANN, M. A., AND EDWARDS P. R. (1937). The spontaneous transmission of IV-variants of *Salmonella aertrycke* from pigeons to rabbits. *Amer. Jl. Hyg.*, Vol. 26, No. 1, pp. 135-137.

HOHN, J., AND BECKER, P. (1937). Bakteriologische und Serologische Erfahrungen während der Häufung von Paratyphus-erkrankungen in Essen in Herbst 1926. *Z. bl. f. Bakt.* I, Abt. Orig., Vol. 103, pp. 184-200.

HOHN, J., UND HERMANN (1936). Der Kulturtyp der Erreger der Typhus-Paratyphus-Gruppe und seine Bedeutung für die Standortsgebundenheit. *Z. Hyg.*, Vol. 117, pp. 722-741.

HOLE, N. (1932). Salmonella infections in Ducklings. *Jl. Comp. Path.*, Vol. 45, pp. 161-171.

HORMAECHE, E., PELUPPO, C. A., UND ALEPPO, P. L. (1937). Zur Ätiologie der Sommerdiarrhoë der Kindren mit besondere Berücksichtigung der Salmonella Infectionen. *Z. Hyg.*, Vol. 119, pp. 453-458.

HUBENER (1909). Ueber Paratyphus C Bacillen als Erreger akuter gastroentritis. *Med. Clinik*, 1909. Cited by Uhlenhuth und Haendel.

HUBENER, E. (1910). Fleischvergiftungen und Paratyphus-infektionen, ihre Entstehung und Verhütung. Verlag von Gustav Fischer, Jena.

HUSGEN, H. (1931). Zum Vorkomen der *Bact. enteritidis breslaviense* bei geschlachteten Gänsen. *B.T.W.*, Vol. 47, pp. 673-675.

HUTCHEON, D. (1893). Liver disease in Calves. *Ann. Rep. Col. Vet. S.* Cape of Good Hope, p. 4.

JANSEN, J. (1934a). Tuberculose en Paratyphus by Eenden en eenige andere Eendeziekten. *Tydsch. v. Diergen*, Vol. 61, pp. 1025-1031.

JANSEN, J. (1934b). Paratyphus by Eenden. *Tydsch. v. Diergen*, Vol. 61. pp. 1247-1254, p. 1309.

JANSEN, J. (1935). Agglutinatiemethode by Eenden. *Tydsch. v. Diergen,* Vol. 62, pp. 517-526.

JANSEN, J. (1936). Eendenkuikensterfte door *S. typhi-murium* en *S. enteritidis* var. Essen. Ibid., Vol. 63, pp. 140-142.

JENSEN, C. O. (1891). *Maanedskr. f. Dyrlaager.* Vol. 4, p. 140. Quoted by Jensen (1913).

JENSEN, C. O. (1905). *Zeitsch. f. Tiermed.*, Vol. 9 [cited by Jensen (1913)].

JENSEN, C. O. (1913). Kälberruhr. *Handbuch der Pathogenen Mikroorganismen (Kolle u. Wassermann).* Vol. 6, pp. 121-144.

JOEST (1906). Bericht über die Tierarztl. Hochschule, Dresden, 1906. Quoted by von Heelsbergen.

JONES, E. R., AND WRIGHT, H. D. (1936). *Bact. Aertrycke* food poisoning due to contamination of food with excreta of mice. *Lancet,* Vol. 230, pp. 22-23.

JOOS, A. (1903). Untersuchungen über die Verschiedenen Agglutinine des Typhus-serum. *Z. bl. Bakt.*, Abt. I, Orig. Vol. 33, pp. 762-783.

JORDAN, E. O. (1917). The differentitation of the paratyphoid enteritidis Group I. *Jl. Inf. Dis.*, Vol. 20, pp. 457-483.

JORDAN, E. O. (1925). The differentiation of the paratyphoid enteritidis group. *J. Inf. Dis.*, Vol. 36, pp. 309-329.

JORDAN, E. O., AND HARMON, H. (1928). A new differential media for the paratyphoid group. *Jl. Inf. Dis.*, Vol. 42, pp. 238-241.

JORDAN, E. O., AND VICTORSON, RUTH (1917). The differentation of the paratyphoid enteritidis group II. *Jl. Inf. Dis.*, Vol. 21, pp. 554-555.

JOWETT, W. (1908). Epizootic pneumo- pericorditis in the turkey. *Jl. Comp. Path. and Ther.*, Vol. 21, pp. 324-330.

JUNGHERR, E., AND BORDEN, A. J. (1935). Serological types of Salmonella isolated from paratyphoid in chicks. *J. Bact.*, Vol. 32, p. 127.

JUNGHERR, E., AND WILCOX, K. S. (1934). *Salmonella aertrycke* variant as an etiological agent of paratyphoid in pigeons. *Jl. Inf. Dis.*, Vol. 55, pp. 390-401.

KARSTEN (1933). Erfahrungen und Betrachtungen über die Enteritis-Gaertner-Infektion des Rindes. *Arch. f. Wiss. u. Prak. Tierh.* Vol. 66, No. 3, p. 189.

KAUFFMANN, F. (1929a). Zur Paratyphusfrage. *Z. bl. Bakt.*. Ref. Vol. 94, pp. 282-288.

KAUFFMANN, F. (1929b). Der Typus " Berlin " der Paratyphus-C Gruppe. *Z. Hyg.*, Vol. 110, pp. 537-555.

KAUFFMANN, F. (1930a). Neue Serologische Typen der Paratyphus Gruppe. Ibid 111, pp. 221-232.

KAUFFMANN, F. (1930b). Der Antigen-Aufbau der Typhus-Paratyphus-Gruppe. *Z. Hyg.*, Vol. 111, pp. 233-246.

KAUFFMANN, F. (1930c). Die Kulturelle Differential-diagnose der Typhus-Paratyphus-Gruppe. Ibid 111, pp. 247-255.

KAUFFMANN, F. (1934). Untersuchungen ueber die Duisberger Gallinarum-Stämme. *Z. bl. Bakt.* 1, Orig., Vol. 132, pp. 337-342.

KAUFFMANN, F. (1935a). Ueber Serologische und Kulturelle Varianten der Paratyphus-B und Mäusetyphus-Bacillen. *Z. Hyg.*, Vol. 116, pp. 368-384.

KAUFFMANN, F. (1935b). Über einen neuen Serologischen Formenwechsel der Typhusbacillen. *Z. Hyg. Infektkr.*, Vol. 116, pp. 617-651.

KAUFFMANN, F. (1935c). Über die Typeneinteilung der Gaertner-Gruppe. *Z. Hyg.*, Vol. 117, pp. 431-450.

KAUFFMANN, F. (1936a). Untersuchungen über die Körperantigene der Salmonella-Gruppe. (v-w). *Z. Hyg.*, Vol. 117, pp. 778-791.

KAUFFMANN, F. (1936b). Ueber einen besonderen Phasen-weschsel bei zwei neuen Salmonellatypen. *Z. Hyg.*, Vol. 118, pp. 540-543.

KAUFFMANN, F. (1936c). Ueber die diphasische Natur der Typhusbacillen. *Z. Hyg.*, Vol. 119, pp. 103-118.

KAUFFMANN, F. (1937). Salmonella-Probleme. *Z. Hyg.*, Vol. 120, No. 2, pp. 177-197.

KAUFFMANN, F., and BURON, F. A. (1935). Kulturelle Untersuchungen in der Salmonella-Gruppe mit besonderer Berücksichtigung der Organischen Säuren. *Z. Hyg.*, Vol. 117, pp. 650-661.

KAUFFMANN, F., u. MITSUI, Ch. (1930). Vergleichende Untersuchungen in der Typhus-paratyphus Gruppe. *Z. Hyg. Infektkr.*, Vol. 111, pp. 749-772.

KAUFFMANN, F., und SILBERSTEIN, W. (1934). Untersuchungen über einige neue Salmonella-Typhen. *Z. bl. f. Bakt.* Orig. 1., Vol. 132, pp. 431-436.

KAUFFMANN, F. und TESDAL, M. (1937). Ueber Zwei neue Salmonellatypen mit α-β phasen-wechsel. *Z. Hyg.*, Vol. 120, No. 2, 168-176.

KHALIFA, ISMAIL ABU BAKR (1935). Serological study of paratyphoid in Pigeons. *Jl. A.V.M.Ass.*, Vol. 39, pp. 24-27.

KILBORNE, F. L. (1893). An outbreak of abortion in mares. *Bull. No. 3, U.S. Dept. Agric.*, pp. 43-52.

KINLOCH, J. P., SMITH, J., and TAYLOR, J. S. (1926). The Aberdeen outbreak of Milkborne Gaertner enteritis, July, 1925. *Jl. Hyg.*, Vol. 25, pp. 434-443.

KLIMMECK (1936). Erfahrungen mit den planmässigen Bekampfung der Enteritis in den Rinderbeständen, *D.T.W.*, Vol. 44, pp. 859-860.

KNOTH, M. (1936). Typendiffentierung an 561 Gaertnerstämmen aus Schlachttieren. *Z. b. Bakt.* 1. orig., Vol. 136, pp. 441-444.

KÖBE, K. (1930). Die Pathagenität des *Bact. suipestifer* für Menschen. D.T.W., Vol. 38, pp. 372-374.

KOLBE, F. (1930). Breslaubakterien in Gänselfleisch. *D T.W.* No. 46. No. 3, pp. 43-45.

KRUGER, H. (1932a). Ueber den Nachweis des *Bact. suipestifer* bei einen typhusähnlicher Erkrankung des Menschen. *Z. bl. f. Bakt.* orig., Vol. 123, pp. 416-425.

KRUGER, H. (1932b). Die Bedeutung des *Bact. suipestifer* als Infektionserreger für den Menschen. *Z. bl. f. Bakt.* orig., Vol. 123, pp. 425-434.

KRUMWIEDE, C., PROVOST, D. J., and COOPER, G. M. (1922). Studies in the paratyphoid group VII. Enteric infection (food poisoning) due to tapioca pudding contaminated with *B. cholerae-suis*. *Jl. Med. Res.*, Vol. 43, pp. 53-59.

KRUMWIEDE, C., COOPER, G., and PROVOST, D. J. (1925). Agglutinin absorption. *Jl. Immunol.*, Vol. 10, pp. 55-239.

KRUSE, W. (1896). Bacillen-*Bacillus Suipestifer Flugge Mikro-organismen*, Band 2. Verlag von Vogel, Leipsig, pp. 401-404.

KUPPELMAYR (1925). Zur Kasuistik der Fleisch-vergiftungen. *Z. Fleisch. u. Milchhyg.*, Vol. 34, pp. 181-186, pp. 195-198, pp. 213-215.

KUTTNER, A. G. and ZEPP, H. D. (1932). Paratyphoid-like fever in children due to the *Salmonella suipestifer* group. *Bull. John Hopkins Hosp.*, Vol. 51, pp. 373.

KUTTNER, A. G., and ZEPP, H. D. (1933). *Salmonella suipestifer* infection in Man. *J.A.M.Ass.*, Vol. 101, pp. 269.

LANGE und PRSZLER (1926). Bericht ueber zwei Fleischvergiftungsepidemien in Bezirk St. in Jahre 1924. *Z. Fleisch, und Milchhyg.*, Vol. 36, pp. 177-180.

LASCHTSCHENKO, P. (1909). Ueber die Keimtötende und entwicklungshemmende Wirkung von Hühnereiweiss. *Z. Hyg.*, Vol. 64, pp. 419-426.

LECOQ (1906). Quoted by Scott 1930.

LEE, C. D., HOLM, G. and MURRAY, C. (1936). Paratyphoid infection in turkeys. *Jl. A.V.M. Ass.*, Vol. 42, pp. 65-76.

LEHR, E. (1927). Ueber eine durch den *Bac. enteritidis* Gaertner hervorgerufene Seuchenhafte Darmentzündung in einen Rinderbestende. *B.T.W.*, Vol. 43, pp. 274-430.

LEHR, E. (1928). Ueber das Vorkommen von echten Paratyphus-B-Schottmüller-Bazillen bei Tieren. *Z. bl. Bakt.*, Vol. 107, pp. 69-83.

LERCHE (1936). Zur Entstehung bakterieller Lebensmittelschädungen durch Geflügel und Geflügelprodukte. *D.T.W.*, Vol. 44, pp. 531-533.

LESBOUYRIES, DADOT et BERTHELOU (1933). Avortement paratyphique de la Brebis. *Bull. Acad. Vet. Fr.*, Vol. 6, pp. 318-321.

LESBOUYRIES ET VERGE (1932). Paratyphose du Pigeon. *Bull. Acad. Vet. de France.* Vol. 5, pp. 294-301.

LOVELL, R. (1931). A member of the Salmonella group causing abortion in sheep. *J. Path. and Bact.*, Vol. 34, pp. 13-22.

LOVELL, R. (1932a). The Salmonella group of bacteria. *Bul. Hyg.*, Vol. 7, No. 7, pp. 405-415.

LOVELL, R. (1932b). The relation of meat and meat products to bacterial food poisoning in man. *Vet. Rec.*, Vol. 12, pp. 1052-1059.

LUTJE (1924). Ein Weiter Beitrag zum Kapital der Paratyphusinfektionen. *D.T.W.*, Vol. 32, pp. 142-150, 157-195.

LUTJE (1926). Paratyphuserkrankungen verursacht durch das *B. enteritidis* Gaertner bei erwachsenen *Rindern*. *D.T.W.*, Vol. 34, pp. 437-442, 453-458.

LUTJE (1930). Paratyphuserkrankungen der Haustiere. Stang-Wirth: *Tierheilk. u. Tierz.*, Vol. 7, pp. 655-681.

LUXWOLDA (1913). De Bakterien-flora van het Vleesch en de Vleeschlymphklieren by 62 kalveren met septicaemische verschynselen. *Tydschr. v. Veeartsenykunde.* Vol. 40. Cited by Clarenburg.

LANDSTEINER, K. and LEVINE, P. (1932). On the Forrsman antigens in *B. paratyphosus B on B. dysenteriae, Shiga.* *Jl. Immun.*, Vol. 22, pp. 75-82.

MACADAM, W. (1919). An account of an infection in Mesopotamia due to a bacillus of the Gaertner-paratyphoid group. *Lancet* 1919. Vol. 2, p. 189.

MACFADYEAN, J. (1893). Epizootic pneumo-pericarditis in the turkey. *Jl. Comp. Path. and Ther.*, Vol. 6, pp. 334-345.

MACFADYEAN, J. and EDWARDS, J. T. (1917). Contagious abortion in mares and joint-ill in foals: Etiology and serum treatment. *Jl. Comp. Path.*, Vol. 30, pp. 321-366.

McGAUGHEY, C. A. (1933). Bacteria of the enteric group among poultry. *Vet. Jl.*, Vol. 88, p. 16.

MACKIE, F. P. and BOWEN, G. J. (1919). Notes on the chracters of an anomalous member of the paratyphoid group met with in Mesopotamia. *J.R.A.M.C.*, Vol. 33, p. 154.

MAGNUSSON, H. (1919). Joint-ill in foals: Etiology. *Jl. Comp. Path.*, Vol. 32, pp. 143-182.

MANNINGER, R. (1918). Paratyphus des Wassergeflügels. *Allat. Lapok.*, Vol. 25, (1918). *B.T.W.*, Vol 35, (1919), p. 98.

MARTINAGLIA, G. (1929). Diseases in domestic animals in South Africa due to organisms of the Salmonella group. *15th Rep. D.V.S.*, pp. 233-295.

MASON, J. H. (1933). A new culture tube. *Jl. S.A.V.M. Ass.*, Vol. 4, No. 2, pp. 1-2.

MASON, J. H. (1936). The isolation of single bacterial cells. *Onderstepoort Jl. of Vet. Sc.*, Vol. 7, No. 1, pp. 133-142.

MATERNA, A., and JANUSCHKE, E. (1925). Ein Beitrag zur der Frage den Aetiologischen Beziehungen zwischen der Bazillären Schweinepest und der Paratyphus β des Menschen. *Z. f. Fleisch. u. Milchhyg.*, Vol. 35, pp. 298-300.

MATTLET, G. (1932). Quelques considerations sur des cas de Fievre paratyphoide C. et contribution a l'etude des Bacilles paratyphoides C. *An. Soc. Belg. Med. Trop.*, Vol. 11, pp. 455-478.

MAZZA, C. (1899). Bakteriologische Untersuchungen über einer neuerdings aufgetretene Hühnerepizootie. *Z. bl. Bakt.*, Vol. 26, pp. 181-185.

MEYER, R. (1933). Die Bedeutung des Geflügels für die Enstehung von Lebensmittel-vergiftungen. *Z. Fleisch. und Milchhyg.*, Vol. 44, No. 5, pp. 81-83.

MEYER, R. (1934). Was lehrt die Statistik der Fleischvergiftungen der letzten 10 Jahre? *B.T.W.*, Vol. 50, pp. 213-215.

MEYER, R. (1936). Zur Statistik der Fleischvergiftungen in den Jahren 1933-1934. *Z. Fleisch. u. Milchhyg.*, Vol. 46, pp. 62-65. pp. 83-86.

MEYER, K. F. and BOERNER, F. (1913). Studies on the etiology of epizootic abortion in mares. *Jl. Med. Res.*, Vol. 29, p.325. *Ellenb. Jhr.*, Vol. 33, p. 96. (MacFadyean and Edwards.)

MEYER, K. F., TRAUM, J. and ROADHOUSE, C. L. (1916). The *Bacillus enteritidis* as the cause of infectious diorrhoea in Calves. *J.A.V.M.A.*, Vol. 2, pp. 17-35.

MIESSNER (1926). Zur Diffentierung der Bakterien der Paratyphus-Enteritis-Gruppe. *Z. bl. Bakt.*, (Orig.), Vol. 97, pp. 242-264.

MIESSNER, H. u. BAARS, G. (1927). Zur Typendifferenzierung der Bakterien der Paratyphus-Enteritisgruppe. *D.T.W.*, Vol. 35, pp. 639-648.

MIESSNER und BERGE (1917). Der *Paratyphus abortus-equi* als Ursache des Seuchenhaften Verfohlens in Deutschland. *D.T.W.*, Vol. 25, No. 2. (Copy not available.) Quoted by Magnusson.

MIESSNER und KOHLSTOCK (1912). Croupöse Darmentzündung beim Rinde, Verursucht durch den Bacillus enteritidis Gaertner. *Z. bl. f. Bakt.*, I abt. orig., Vol. 65, pp. 38-41.

MIESSNER, H. und KOSER, A. (1934). Entenei und Lebensmittelvergiftungen. *D.T.W.*, Vol. 42, pp. 717-720.

MOHLER, J. R. and BUCKLEY, J. S. (1902). Report on an enzootic among cattle caused by a bacillus of the enteritidis group. *19th Rep. B.A.I.*, pp. 297-331.

MOORE, V. A. (1895). On a pathogenic bacillus of the hog-cholera group assoiciated with a fatal disease in pigeons. *Bur. An. Ind., U.S.A.*, Bull. No. 8, pp. 71-78.

MOULIN et AMICHAU (1911). Recherches sur les Affections Typhoides du Cheval. *Compt. Rend. Soc. de Biol.*, Vol. 70, pp. 75.

MULLER (1921). Paratyphus beim Pferde als Ursache von Massenerkrankungen beim Menschen nebst Bemerkungen ueber der Schuldfrage des Tierarztes bei Massenerkrankungen nach dem Genuss von als tauglich begutachtetem Fleische. *Z. Fleisch. u. Milchhyg.*, Vol. 31. pp. 237-245.

MULLER, O. und RODENKIRCHEN, I. (1933). Ueber Lebensmittelvergiftungen durch *Bact. enteritidis Gaertner*. *Veröff. Med. Verw.*, Vol. 39, H. 3, Berlin. Quoted by Meyer.

MURRAY. C. (1919). The cause of abortion in mares. *Jl. Inf. Diseases*. Vol. 25, pp. 241-248.

MURRAY, C. (1934). The classification of the paratyphoid diseases. *12th Intern. Vet. Congress*, Vol. 2. pp. 230-248.

NABARRO, D., WHITE P. BRUCE, DYKE, S. C., and SCOTT, W. M. (1929). Two cases of human infection by the American hog-cholera bacillus. *Lancet.*, Vol. 217, pp. 868-870.

NEUKIRCH, P. (1918). Ueber Menschlichen Erkrankungen durch Bazillen der Glasser-Valdagsen-Gruppe in der Turkei. *Z. f. Hyg.*. Vol. 85. p. 103.

NEWSON, I. E. and CROSS, F. (1924). An outbreak of paratyphoid dysentery in lambs. *Jl. A.V.M.A.*, Vol. 19, No. 3, pp. 289-300.

NEWSON, I. E. and CROSS, F. (1930). Paratyphoid dysentery in lambs again. *Jl. A.V.M. Ass.*, Vol. 29, No. 1, 91-92.

NEWSON, I. E. and CROSS, F. (1935). Third outbreak of paratyphoid dysentery in lambs. *J.A.V.M. Ass.*, Vol. 39. pp. 534-536.

OBICH (1865). *Wochenschr. f. Tierheilk. u. Viehzucht.*, (Cited by C. O. Jensen).

O'BRIEN, R. A. (1910). Guinea pigs as chronic carriers of an organism belonging to the food-poisoning group. *Jl. Hyg.*, Vol. 10. pp. 231-236.

ORCUTT, M. (1924a). Flagellar agglutinins. *Jl. Exp. Med.*. Vol. 40, pp. 43-49.

ORCUTT, M. (1924b). The effect of heat on flagellar and somatic agglutination. Ibid., Vol. 40. pp. 627-632.

PALLASKE, G. (1930). *Archiv. Tierhielk.*, Vol. 62, pp. 89. (Quoted by Schaaf.)

PALLASKE, G. und LOMMETSCH. R. (1933). Beitrag zur Frage der Beziehungen des anatomischen Befundes zum bakteriologischen Untersuchungs-ergebnis beim sog. Kälberparatyphus. *Z. f. Fleisch und Milchhyg.*, Vol. 43, pp. 102-106.

PFAFF (1921). Eine Truthühnerseuche mit Paratyphus-Befund. *Z. f. Infek. Krankh. d. Haust.*, Vol. 22, pp. 285-298.

PFEILER, W. (1911). Ueber ein Seuchenhaftes durch Bakterien aus der Paratyphus-gruppe verursachtes Kanariensterben. *B.T.W.*, Vol. 27, pp. 953-954.

PFEILER, W. (1919). Ueber Paratyphus-B. Bazillen-Befund bei einer Junggens nebst Allgemeinen Bemerkungen über das Vorkommen von Paratyphaceen beim Geflügel und bei Vögeln. *Z. Fleisch. und Milchhyg.*, Vol. 29, pp. 115-117, p. 130.

PFEILER, W. und REISE, A. (1913). Ueber das Vorkommen von Bakterien aus der Gruppe der Fleischvergifter bei Vögelen. Paratyphus-B Infektion bein Huhn, *Z. bl. f. Bakt.* I. Orig., Vol. 68, pp. 174-181.

POELS (1899). Rapport over de Kalverzucht in Nederland's Gravenshage.

PREISZ, H. (1898). Aetiologische Studiën über Schweinespest und Schweineseptikaemie. *Z. f. Tiermed.*, Bd. 2. HI Pl. Cited by H. Bischoff. *Z. bl. Bakt.*., Vol. 23, pp. 666-667.

PRESSLER (1930). Fleischvergiftung nach Genuss von Gans-Leberpastete. *B.T.W.*, Vol. 46, No. 50, pp. 961.

PRÖSCHOLDT, O. (1931). Gaertnerinfektionen der Kalber und Rinder. *Z. f. Inflr. (Haust.)*, Vol. 39, pp. 115-140.

PYPER, A. (1923). Widal versus complement-Fixation. *S.A. Med. Rec.*, February 10th and 24th.

RAVITCH, M. M. and WASHINGTON, J. A. (1937). Suipestifer septicaemia and meningitis complicating meningococci septicaemia and meningococcic meningitis. *J.A.M. Ass.*, Vol. 109, No. 14, pp. 1122-1123.

REINHOLD, W. (1912). Infektionversuche mit den Fleischvergiftern, *Bac. enteritidis* Gaertner und *Bac. paratyphus-B*, beim Geflügel. *Z. bl. Bakt.*, Abt. I orig., Vol. 62, pp. 312-334.

REITSMA (1924). *Tydsch v. Vergel. Geneeskunde X.* Quoted by v. Heelsbergen.

RETTGER, L. F. and SPERRY (1912). *Jl. Med. Res.*, Vol. 26, p. 56. (Quoted by T. M. Doyle.)

RETTGER, L. F. and SCOVILLE, M. (1920). *Bacterium anatum* N.S., the etiologic factor in a widespread disease of young ducklings known in some places as "Keel". *Jl. Inf. Dis.*, Vol. 26, pp. 217-229.

RETTGER, L. F., PLASTRIDGE, W. N. and CAMERON, R. (1933). Endemic paratyphoid infection in Turkeys. *Jl. Inf. Dis.*, Vol. 53, No. 2, p. 272.

RIEVEL (1933). Versuche an Rindern die Dauerausscheider von Enteritidis-Gaertner-Bakterien met dem Kote Sind. *Arch. Wiss. Prakt. Tierh.*, Vol. 66, pp. 317-332.

RIMPAU, W. (1937). Ueber Infektionen das Menschen durch Hans und Stalltiere. *Munch. Med. Wsch.*, Vol. 84, pp. 413-414. Abstr. *Vet. Rec.* Vol. 50, No. 7, p. 185.

ROBINSON, E. M. and MARTINAGLIA, G. (1932). An outbreak of paratyphoid in pigs. *Jl. S.A.V.M. Ass.* June 1932.

SAHAYA, J. and WILLIEMS, H. (1927). A disease in pigeons due to a Salmonella. *Jl. Comp. Path. and Ther.*, Vol. 40, pp. 305-308.

SALMON, D. E .(1886). Investigations in swine plague. *2nd Report of Animal Industry*, pp. 184-246.

SALMON, D. E. (1886). Investigation of swine diseases. *3rd Rep. Bureau of Animal Industry*, pp. 20-75.

SALMON, D. E. (1886). Swine plague. Ibid. 75-100.

SALMON, D. E. (1904). Infectious enteritis in pigeons. *21st Ann. Rep. B.A.I., U.S.A. Dept. Agric.* 1904, p. 29.

SALMON, D. E., and SMITH, T. (1885). *1st Rep. Bureau Animal Industry.*

SALMONELLA Sub-committee of the Nomenclature Committee of the International Society for Microbiology (1935). *J. Hygiene,* Vol. 34, pp. 333-350.

SAVAGE, W. G. (1909). Further report upon the presence of the Gaertner group of organisms in the animal intestine. *37th Ann. Rep. Local. Gov. Board* (1907-1908), H.M. Stat. Office., pp. 425-444.

SAVAGE, W. G. (1912). A note on the inter-clissification of the Gaertner. *J. Hyg.,* Vol. 12, pp. 1-4.

SAVAGE, W. G., and WHITE P. BRUCE (1925). An investigation of the Salmonella group with special reference to foodpoisoning. *M.R.C. Spec. Rep. Series* 91, H.M. Stationery Office.

SCHAAF, J. (1933). Zur infektiösen Enteritis der Enten. *Z. bl. Bakt.* I orig., Vol. 128, pp. 519-530.

SCHAAF, J. (1936). Die Salmonellose (Infektiöse Enteritis, Paratyphose des Geflügels, irher Bedeutung und Bekämpfung. *Z. Infek. Krank. Haust.,* Vol. 49, pp. 322-332.

SCHALM, O. W. (1937). Study of a paratyphoid infection in chicks. *Jl. Inf. Dis.,* Vol. 61, pp. 208-216.

SCHERMER u. EHRLICH (1921). Weitere Beiträge ueber die Paratyphuserkrankungen der Haustiere. *B.T.W.,* Vol. 37, pp. 469-437.

SCHIFF, F. (1923). Weitere Untersuchungen über den Rezeptorenapparat in der Paratyphus-Gruppe. *Z. f. Immu. Forsch.,* Vol. 35, p. 293.

SCHMITT, F. M. (1908). Zur Aetiologie Seuchenhaften Kälber-Sterbens. *D.T.W.,* Vol. 16, No. 47, pp. 673-676.

SCHMITT, F. M. (1908). Der Bacillus Paratyphus B als Krankheits Erreger bei Kälbern. Ibid., pp. 685-689.

SCHOFIELD (1914). The etiology of pyemic arthritis in foals. *Jl. Inf. Dis.,* Vol. 15, pp. 409-416.

SCHOLTEN, R. Th. (1936). The resistance developed against bacteriophage. *Jl. Hyg.,* Vol. 36, pp. 452-455.

SCHOTTENMULLER, H. (1901). Weitere Mitteilungen über Mehrere das Bild des Typhus bietende Krankheitsfälle, hervorgeruten durch Typhus ähnlichen Bacillen (Paratyphus). *Z. Hyg. Infekt. Kr.,* Vol. 34, pp. 368-396.

SCHUTZE, H. (1920). The paratyphoid B group. *Lancet,* Vol. 198, pp. 93-97.

SCHUTZE, H. (1922). The permanence of the serological paratyphoid B types, with observations on the non-specificity of agglutination with "rough" variants. *Jl. Hyg.,* Vol. 20, pp. 330-341.

SCOTT, W. M. (1926a). The Thompson type of Salmonella. *Jl. Hyg.,* Vol. 25, p. 398-405.

SCOTT, W. M. (1926b). Food-poisoning due to *Bacillus suipestifer* Ibid., Vol. 25, pp. 406-414.

SCOTT, W. M. (1930). Food-poisoning due to eggs. *B.M.J.,* 1930, pt. 2, pp. 56-58.

SCOTT, W. M. 1930). Consequences of Salmonella infections of eggs. *Jl. Path. and Bact.,* Vol. 35, p. 655.

SCOTT, W. M. (1934). Identification of Salmonella Types. *Ann. Rep. Chief Medical Officer of Ministery of Health* for 1933, pp. 189-192.

SEYMOUR, R. T. (1936). Pyosepticaemia of foals due to *B. abortivo equinus. Vet. Bull. U.S. Army,* Vol. 30, pp. 277-282. (Original not seen).

SHIRLAW, J. F. (1925). Pyosepticaemia of calves. *Indian Jl. of Vet. Sc.,* Vol. 5, pp. 232-250. *Vet. Bul.,* Vol. 6, No. 6, p. 533.

SHIRLAW, J. F. and IYER S. GANAPATHY (1937). A note on a variety of S. enteritidis from pigeons. *Indian Jl. of Vet. Sc. and An. Husb.*, Vol. 7, No. 2, pp. 231-242.

SLADDEN, A. F. and SCOTT, W. M. (1927). Food-poisoning due to bacilli of the type *B. morbificans-bovis* (Basenau). *Jl. Hyg.*, Vol. 26, 111-117.

SILBERSTEIN, W. (1931). Neue kulturelle Methoden zur Differentialdiagnose in der Paratyphusgruppe. *Z. bl. Bakt.* 122, Beihaft, pp. 131-135.

SMITH, T. and MOORE, V. A. (1894). Additional investigation concerning infectious swine diseases. *B.A.I.*, Bull. No. 6.

SMITH, J. (1934). Sporadic salmonella infections: A new salmonella type. *Jl. Hyg.*, Vol. 34, pp. 357-360.

SMITH, J., and SCOTT, W. M. (1930). A continued fever due to a Gaertner-like salmonella of the "Dublin" type. *Jl. Hyg.*, Vol. 33, No. 1, pp. 32-39.

SMITH, T. (1893). On a pathogenic bacillus from the vagina of a mare after abortion. *B.A.I., U.S. Dept. Agric.*, Bull. 3, pp. 53-59.

SMITH, T. (1893). Abortion in mares. *8th and 9th Report B.A.I., U.S. Dept.*м *Agric.*, pp. 65-66.

SMITH, T. and MOORE, V. A. (1894). Arbeiten aus dem pathologischen Laboratorium des Bureau of Animal Industry Washington, U.S.A., *Z. bl. f. Bakt.*, Vol. 16, pp. 231-241.

SMITH, TH. and REAGH, A. L. (1903).. The non-identity of agglutinins acting upon the flagella and upon the body of bacteria. *Jl. Med. Res.*, Vol. 10, p. 89.

SMITH, T. and TENBROECK (1915a). Agglutination affinities of a pathogenic bacillus from fowls (fowl typhoid). (*Bacterium sanguinarium*, Moore) with typhoid bacillus of man. *Jl. Med. Res.*, Vol. 31, p. 503.

SMITH, T. and TENBROECK, C. (1915b). A note on the relationship between *B. pullorum* (Rettger) and the fowl typhoid bacillus (Moore). *Jl. Med. Res.*, Vol. 31, p. 547.

SPRAY, R. S. and DOYLE, L. P. (1921). Paratyphoid bacilli from chicks. *Jl. Inf. Dis.*, Vol. 28, pp. 43-47.

SPRINGER (1911). Ein Fund von *Bacillus Paratyphi*, Typus A, in der Gallenblase, nebst Einwirkung der Bakterien der Typhus-coli-gruppe auf verschiedene Zuckerarten. *Z. bl. f. Bakt.*, Vol. 60, pp. 2-14.

STANDFUSS, R. (1925). Erfahrungen ueber das Vorkommen von Keimen aus Paratyphus-Enteritidis-Gruppe bei Notgeschlachteten Tiere. *Z. Fleisch. u. Milchhyg.*, Vol. 36, pp. 43-44.

STANDFUSS, R. u. WILKEN, H. (1933). Ueber die Ausscheidung van Enteritis-Bakterien durch die Milch und deren Nachweis bei Rindern. *D.T.W.*, Vol. 41, pp. 721-726.

STEPHAN, J. u. GEIGER, W. (1922). Paratyphusbakterien als Ursache Seuchenhaften Verlammens. *D.T.W.*, Vol. 30, pp. 512-513.

STERN, W. (1916). Studien zur Differentierung der Bakterien der Coli-Typhus-Gruppe mittels gefärbter, flüssiger Nährboden. Beitrage zur Biologie der Bakteriengruppe Paratyphus-B. *Z. bl. Bakt.*, Vol. 78, pp. 481-492.

STROZZI, P. (1931). *La Clin. Vet.*, Vol. 54, p. 927. Quoted by Schaaf.

STROZZI, P. (1934). Il paratifo dei vitelli in Italia. *Clin. Vet. Milano*, Vol. 57, pp. 337-354. *Vet. Bull.*, Vol. 6. p. 639.

TEN BROECK, C. (1920a). A group of paratyphoid bacilli from animals closely resembling those found in man. *J. Exp. Med.*, Vol. 32, p. 19.

TEN BROECK, C. (1920b). Bacilli of the hog cholera group (*Bacillus cholerae-suis*) in man. Ibid. Vol. 32, pp. 33-40.

TEN BROECK, C., LI, C. P. and YII, H. (1931). Studies in paratyphoid C bacilli isolated in China. *Jl. Exp. Med.*, Vol. 53, pp. 307-315.

TESDAL, M. (1936). Ein neuer Salmonellatypus (*S. hvittingfoss*). *Z. Hyg.*, Vol. 118, pp. 533-539.

TESDAL, M. (1937). Ein neuer Salmonellatypus (*S. oslo*). *Z. Hyg.*, Vol. 119, pp. 451-452.

THOMASSEN, M. (1896 en 1897). Bacteriaemie met haematogene nephritis, urocystitis (Bacteriurie) der Kalveren. *Tydsch. v. Veeartsenykunde en Veeteelt.*, Vol. 23, p. 335 en Vol. 24, p. 307. (Quoted by Clarenburg.)

THOMASSEN, M. (1899). Une nouvelle septicemie des veaux. *Ann. Inst. Pasteur.*, Vol. 11, p. 523. (Quoted by Jensen.)

TITZE, C., und WEICHEL, A. (1908). Die Aetiologie der Kälberruhr. *B.T.W.* Vol. 1908, No. 26, pp. 457-458.

UHLENHUTH, P. (1926). Paratyphus. 11. Tagung der deutschen Vereinigung für Mikrobiologie 1925. *Z. bl. f. Bakt.*, I. orig., Vol. 97, pp. 219-241.

UHLENHUTH, P. und HAENDEL, L. (1913). Schweinepest und Schweineseuche. *Handbuch der Pathog. Mikro-organismen*, Band 6, No. 2, pp. 325-434.

UHLENHUTH und HUBENER (1909). Weitere Mitteilungen über Schweinepest mit besonderer Berücksichtigung der Bakteriologie der Hogcholeragruppe. *Z. bl. f. Bakt.*, Vol. 42, (ref.), Beiheft. 127 (Andrewes and Neave).

URBAIN, STOCAIME ET CHAILLOT (1929). Etude d'une épidèmie Paratyphique-B chez le cheval. Essais de Vaccination. Ref. Ellenberger-Schutz. *Jber.*, Vol. 49, p. 875.

VAN HEELSBERGEN, T. (1914). Abortus bei Stuten durch einen Paratyphus-B-Bacillus. *Z. bl. Bakt.*, Vol. 72, pp. 38-69.

VAN HEELSBERGEN, T. (1929). Handburch der Geflügelkrankheiten und Geflügelzucht. Ferd. Enke. Stuttgart.

VILJOEN, P. R. and MARTINAGLIA, G. (1926). The occurrence of paratyphoid, *Bacillus enteritidis*, infection in calves in South Africa. *S.A. Jl. of Sc.*, Vol. 23, pp. 532-544.

VILJOEN, P. R. and MARTINAGLIA, G. (1928). Preliminary report on investigation carried out in connection with a calf disease in the Marico district, with special reference to paratyphoid, *B. enteritidis* infection. 13-14 *Rep. Div. Vet. Educ. Res.*, pp. 535-549.

WARNECKE, H. (1914). Het voorkomen van den *Bacillus enteritidis* Gaertner, by Kalveren. *Tydsch. v. Veeartsenykunde.*, Vol. 41, pp. 357-359.

WARRACK, G. H. and DALLING, T. (1933). Salmonella infections in young ducklings and duck's eggs. *Vet. Jl.*, Vol. 89, pp. 483-487.

WASSEN, A. (1935). Essais di' application au vibrion cholerique de la Méthode fondée sur la faculte de deplacement des bactéries. *Bull, mens. off. intern. hyg. publ.*, Vol. 25, pp. 1-14.

WEBER (1936). Zur Dauerausscheiderfrage in Rindviehbeständen. Bekämpfung der Enteritis als Folge einer Infektion mit *Bact. enteritidis* (Gaertner) *breslaviense* oder Paratyphus-B (Schottmüller), *B.T.W.*, Sept. 11, 1936, pp. 599-601.

WEIL, E. (1917). Paratyphus-B-ählicher Krankheitserreger (Typus suipestifer Voldagsen) in Albanien. *Wiener Klin. Wsch.*, Vol. 30, p.1061. (Cited by Bruce White and Andrewes and Neave.)

WEIL, E. and FELIX. A. (1917). *Wien. Klin. W. Schr.*, Vol. 30, p. 1509. (Quoted by Bruce White, 1929.)

WEIL, E. und FELIX, A. (1920). Ueber den Doppeltypus der Reseptoren in der Typhus-paratyphus-Gruppe. *Z. Immun. Forsch.* Tll orig., Vol. 29, pp. 24-91.

WEIL, E., FELIX und MITZENMACHER, F. (1918). *Wien. Klin. Wsch.*, Vol. 31, p. 1226. (Quoted by Arkwright 1931.)

WEIL, E. und SAXL, P. (1917). Ueber eine Infektionskrankheit, bedingt durch einem Keim aus der Paratyphus-Gruppe. *Wien. Klin. Wsch.*, Vol. 30. p. 519. (Andrewes and Neave, and Bruce White.)

WEISGERBER, und MÜLLER. C. (1922). Untersuchungen über eine Seuchenhafte Erkrankung der jungen Gänse in der Provinz Ostpreuszen mit Paratyphus befund. *D.T.W.*, Vol. 30. pp. 663-665.

WHITE, P. BRUCE (1925). An investigation of the Salmonella group, with special reference to food-poisoning. *Spec. Rep. Series M.R.C.*, No. 91, pp. 1-88.

WHITE, P. BRUCE (1926). Further studies of the Salmonella group. *Spec. Rep. Series M.R.C.*, No. 103.

WHITE, P. BRUCE (1929). The Salmonella group. A system of bacteriology. *Med. Res. Council.*, Vol. 4, pp. 86-158.

WHITE, P. BRUCE (1930). Notes on organisms serologically related to S. enteritidis Gaertner I. The Dublin and Tokyo types of Salmonellas. *Jl. Hyg.*, Vol. 29. pp. 443-445.

WILLFUHR, FROMME und BRUNS, H. (1933). Nahrungmittelerkrankungen durch Enteneier. *Veröff a.d. Geb. d. Medizinalverwaltung*. Vol. 39, H. 3. Berlin. (Quoted by Meyer.)

WUNDRAM. G. und SCHONBERG, F. (1933). Neue Untersuchungen von Geschlachteten Mastgänsen auf Fleischvergifter. *B.T.W.*, Vol. 49, pp. 233-235.

ZAHN, E. (1935). Ueber das Vorkommen van "V-varienten" bei Schottmüller und Breslaubakterien. *Z. f. Immunitätsf.*, Vol. 86, pp. 162-164.

ZEUG (1935). Bakterielle Lebensmittelvergiftungen beim Menschen durch Enteneier. *D.T.W.*, Vol. 51, pp. 792-793.

ZINGLE, M. (1914). Untersuchungen uber eine Taubenseuche mit Paratyphus-B-Bazillen-Befund. *Z. Infek. d. Haust.*, Vol. 15, pp. 268-272.

Section IV.

Parasitology.

MÖNNIG, H. O. AND ORTLEPP, R. J. — A tetrachlorethylene emulsion as an anthelmintic.

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Printed in the Union of South Africa by the Government Printer, Pretoria.

A Tetrachlorethylene Emulsion as an Anthelmintic.

By H. O. MÖNNIG and R. J. ORTLEPP, Section of Parasitology, Onderstepoort.

In a previous paper (1936) the authors reported on anthelmintic tests with tetrachlorethylene against the hookworm *Gaigeria pachyscelis* and other nematodes of sheep. In this connection it was mentioned that a mixture of the drug with mineral oil had two distinct disadvantages, viz. that fumes of the drug tended to cause coughing and choking when the sheep were not drenched very carefully and that the rapid absorption of the drug from the digestive tract produced giddiness and anaesthesia. Brief information was further given on attempts to overcome these difficulties by emulsifying the tetrachlorethylene and some success had been obtained with an ordinary soap emulsion.

In this paper it is intended to report on the further development of tetrachlorethylene therapy against the *Gaigeria* hookworm and other worms incidentally affected.

The soap emulsion beforementioned overcame the trouble of coughing and choking, but it was not satisfactory for several reasons. When shaken, this emulsion formed much forth, which led to inaccuracies in its use. It had to be made and diluted with distilled or very soft water. It broke in the abomasum and so did not prevent giddiness due to rapid absorption. A stable emulsion of tetrachlorethylene alone could not be obtained, so that liquid paraffin had to be added, thus increasing costs and volume for transport.

In the further investigation it was attempted roughly to standardise the technique used, in order to obtain a basis for comparison of the different emulsions made, in regard to their physical properties. A stirring apparatus was made, consisting of a vertical shaft carrying two small propellers, of which the lower one produced an upward and the upper one a downward current. The distance between the two propellers on the shaft could be altered. The shaft was revolved at a rate of about 300-400 r.p.m. by a small electric motor taken from an old fan. The speed would naturally vary with the viscosity of the emlusion. The stirrer worked in a glass cylinder which was slightly wider than the width of the propellers.

It is obvious that the apparatus is not comparable in efficiency to modern emulsifying machinery and that a very high dispersion cannot be obtained with it, but it proved to be a satisfactory instrument for preliminary tests.

When an emulsion had been made a small sample was diluted with water on a slide and the size of the particles was measured under the microscope. Another sample was diluted with 4-10 parts of tapwater (which is fairly hard) to test the effect of such dilution on the emulsion and to observe the rate of precipitation on standing. Further samples were mixed with 1 and 1·5 per cent. concentrations of hydrochloric acid. In general the effect of a 1·5 per cent. hydrochloric acid solution on such an emulsion was found to be similar to the effect of fresh abomasal juice of sheep. The effect of the latter itself was also tested on various occasions. Further samples of the emulsion were left standing in order to determine their keeping qualities and samples were also placed in a refrigerator to test the effect of low temperatures.

It is not intended here to present a full report on all the substances tested as emulsifiers and stabilisers, but it should be mentioned that many were tried, including substances now widely used in emulsification, such as sulphonated castor oil, triethynolamine, silicates, etc. The result of these investigations was an emulsion of tetrachlorethylene, made with a resin soap as emulsifier and casein as stabiliser, described below. In arriving at a decision as to the most suitable form of emulsion, the main factors considered were simplicity, cheapness both in manufacture and transport, keeping qualities and above all suitability for the purpose in view as well as efficacy against the parasites.

Resin soaps have been and are being used in the manufacture of many emulsions. They are easy to make and have one particular advantage that they can be diluted with any ordinary water, since the calcium and magnesium resinates are soluble in water.

Casein as stabiliser is also well known. It is cheap, since only small quantities are required, and it can be readily incorporated in the resin soap. As stabiliser for the tetrachlorethylene emulsion it is quite satisfactory and it plays an important part in retarding absorption of the drug, as will be mentioned later.

Resin soap alone does not produce a satisfactory emulsion, because it is broken by acid, although in other ways the emulsion is not a bad one, as is shown by the following example:—

Emulsifier: Water 800 c.c., NaOH 5 gm., Resin 40 gm.

Emulsion: Emulsifier 50 c.c., Tetrachlorethylene 150 c.c. A moderately thick emulsion (pours well but flows slowly).

Particles: Mostly very small, largest measuring 6·5 μ.

Dilution: No change; precipitation 1/60 in first hour in 1:4 dilution.

Acid resistance: 1·5 per cent. HCl breaks emulsion immediately.

Keeping qualities: After 14 days no change, then discarded.

Series of emulsions with resin soap and casein were made in order to determine the most satisfactory proportions of each of the ingredients, particularly in the emulsifier.

Resin.—The quantity of resin used is of no great importance above a certain minimum. Excess of resin, above the minimum, if it is all saponified, will produce a thicker emulsifier and a thicker emulsion, but no important change in the size of the particles. The emulsion, however, soon becomes so thick that it does not flow readily and is therefore unsatisfactory in practice. If not saponified, excess resin is dissolved by the tetrachlorethylene, also producing a thicker emulsion. The quantity of resin determined as optimal in laboratory tests was 5 per cent. of the emulsifier.

Casein.—The optimum quantity of casein corresponds to the quantity of resin used and is also 5 per cent. of the emulsifier. A smaller quantity produces a thinner emulsion and the particle size increases. A larger quantity produces a thicker emulsion without decrease in particle size.

Alkali.—The quantity of alkali must be exactly sufficient to combine with the resin and casein, leaving no excess of free alkali in the emulsifier. Too little alkali will leave unsaponified resin and uncombined casein and produces a thinner emulsion which does not keep well. Excess alkali also produces a thinner emulsion and, although the particle size is also decreased, the emulsion has poor keeping qualities. The correct quantity of alkali would have to be determined for each sample of resin used, as it would vary with the saponification value of the resin. If a highly saponifiable resin is used, the quantity of alkali required for 5 per cent. resin and 5 per cent. casein is equivalent to 0·75 per cent. NaOH, all in relation to the total quantity of the emulsifier. Ammonia and sodium carbonate were tried as alkalis, but there is no particular advantage in using them and consequently NaOH was decided upon.

The emulsifier was eventually made as follows:—To 500 c.c. tapwater, heated to 70° C., is added 6 gm. NaOH and then 40 gm. casein is rapidly stirred in. The casein should contain as little fat as possible and should dissolve readily. The solution is then heated to 85° C. and 40 gm. ground resin is stirred in rapidly. A highly saponifiable resin should be used, such as is used in most soap factories. It should not be ground to a fine powder, else it will tend to form lumps which dissolve slowly. A coarsely ground resin forms no lumps and dissolves readily. The solution should be kept at 85° C. and stirred for about 15-20 minutes until complete combination of the alkali with the other ingredients has occurred. It is then made up to 800 c.c. with cool water.

In the laboratory, with the apparatus described, an emulsion of suitable thickness and maximum particle size of 5 μ is obtained with 25 per cent. emulsifier and 75 per cent. tetrachlorethylene. Samples of such emulsions made over 2 years ago are still keeping well and appear to be unaltered in all respects.

When the process was repeated on a large scale and using a colloid-mill type of emulsifying machine, it was found that

incorporation of air into the emulsion during the process of mixing must be guarded against, since small air-bubbles will hang on in the emulsion and cause it to break after some weeks.

In order to obtain a moderately thick, stable, stock emulsion the tetrachlorethylene has to be added to the emulsifier in successive small quantities while mixing proceeds, and this is especially important in the initial stages. If the requisite quantities of alkali, casein and water are mixed with tetrachlorethylene which contains the correct quantity of resin in solution, emulsification occurs rapidly simultaneously with saponification of the resin. The emulsion, however, does not keep well, probably on account of the fact that saponification is not complete and some alkali remains free.

The emulsion described above can be diluted with any ordinary water. It causes no coughing if it is properly administered. Mixed with 1·5 per cent. hydrochloric acid or abomasal juice, the emulsion does not break, but the casein is precipitated in floccules which, when examined microscopically, appear like a thick emulsion and contain the particles of tetrachlorethylene in the same finely dispersed state in which they existed in the original emulsion. It is probably this reaction which retards absorption to some extent and prevents giddiness, because the tetrachlorethylene will most likely be released gradually from the floccules of casein as the latter become digested.

The emulsion is issued in the concentrated form and is diluted with an equal quantity of water before use. The dose for an adult sheep is 20 c.c. of the diluted emulsion—i.e. 7·5 c.c. tetrachlorethylene—for lambs of 6-12 months old 15 c.c. and for lambs of 3-6 months old 10 c.c. The remedy is given after a preliminary dose of 2·5 c.c. 10 per cent. copper sulphate, so that it should be swallowed into the abomasum. It is not necessary to starve the animals or to keep them from water, but the treament should be carried out in the late afternoon or early morning when it is cool and the animals should be handled quietly.

Experience so far obtained with this emulsion indicates that it is very safe. Tetrachlorethylene is apparently not very harmful. Beyond anaesthesia, from which animals recover if they are left undisturbed when they lie properly, no harmful effects have been noticed. An unsatisfactory emulsion may break, and, if this is administered by an unsuspecting farmer, coughing and choking with ill results may follow, but a good emulsion can be recommended with safety according to present experience.

The emulsion has been found to be effective against *Haemonchus contortus*, *Trichostrongylus* spp., *Nematodirus*, *Gaigeria pachyscelis* and *Bunostomum trigonocephalum* in sheep. It has not been tested against *Ostertagia* because sheep infected with these parasites were not available, but it is quite possible that it may be effective also in this case. Against *Bunostomum* the emulsion is not as effective as against *Gaigeria*, possibly on account of the fact that the former parasite is normally located farther back in the small intestine than

the latter and less of the drug reaches it. Against *Oesophagostomum columbianum* the efficacy is low and very variable on account of rapid absorption of the drug from the small intestine. The addition of 1 c.c. of croton oil to each 10 c.c. of undiluted emulsion increases its efficacy against the nodular worm, but it is very debatable whether this is due to tetrachlorethylene or to the purgative action of the croton oil and whether it is desirable to administer croton oil to sheep suffering from oesophagostomiasis, especially if one considers the condition of their intestines.

Summary.

An emulsion of tetrachlorethylene is described, which is effective against hookworms in sheep (*Gaigeria* and *Bunostomum*) and against *Haemonchus*, *Trichostrongylus* and *Nematodirus*. The emulsion does not cause coughing and choking when it is administered with reasonable care and does not produce giddiness since absorption is somewhat rertarded. The factor of safety is relatively high.

Reference.

ORTLEPP, R. J. and MÖNNIG, H. O. (1936). Anthelmintic tests, chiefly with tetrachlorethylene, for the removal of the hookworm, *Gaigeria pachyscelis* from infested sheep, with observations on the effects of this drug on other parasitic nematodes. *Ond. J. Vet. Sc. An. Ind.*, Vol 7. No. 2, pp. 399-417.

Section V.

Mineral Metabolism and Deficiency.

KELLERMANN, J. H.	Change in body-weight and food consumption of rats on repeated feeding of a deficiency diet.

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Change in Body-Weight and Food Consumption of Rats on Repeated Feeding of a Deficiency Diet.

By

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In his study on the variations in reaction of rats to different diets Bloomfield (1937) observed that when a series of rats of the same breed and of approximately the same age was placed on a defective diet, there were great individual variations in weight loss. Repetitions of the experiments after weight loss had been restored by a normal diet showed that, on the whole, the animals which lost most weight in the first instance did so again and *vice versa*. In a further study French and Bloomfield (1937) stated that " rats which have lost weight as the result of a defective diet and have then been restored to ' normal ' by stock ration show a more rapid weight loss if now placed for a second time on the same defective diet. This ' secondary rapid weight loss ' may occur after as long an interval as 80 days between the first and second periods on defective diet ". The authors, therefore, believe that they have established what may perhaps be called a state of " latent deficiency ". Unfortunately, French and Bloomfield did not measure the food intake and they did not state whether their animals were kept on screens or whether they were allowed access to their excreta.

Inasmuch as South Africa is known for its periodical droughts and adverse seasons, a large percentage of the livestock in this country must sometimes exist for prolonged periods on food probably very deficient in one or more respects. If such periods follow one another in close succession, as is sometimes the case, and if the observation of French and Bloomfield also holds true for farm animals, it is obvious that this phenomenon should be of great importance to the livestock industry in this country. The following experiments were planned, therefore, with the object of further investigating this problem.

Experimental.

Young mature rats of the same breed and of approximately the same age were used in these experiments. They were raised on the colony stock ration used in this laboratory. Before being put on the defective diets, they were fed for one week on the synthetic

(experimental) stock ration used in these experiments. The rats were then fed, in addition to distilled water, diets * that were defective in various respects. The number of rats on each diet varied from 12 to 24 with an even number of males and females in each group. Each rat was housed in a separate cage and, with the exception of experiment IV, kept on a wire screen. The animals were weighed every other day but were fed daily and the total individual food consumption for every two days recorded. The composition of the stock and defective diets is given in Table I.

TABLE I.

COMPOSITION OF RATIONS IN PERCENTAGE BY WEIGHT.

	Colony Stock Diet.*	Experimental Stock Diet.†	Diet low in Vitamins and Minerals.	Diet low in Vitamins, Minerals and Bulk.	Diet low in Proteins.
Yellow maize meal...........	60	—	—	—	—
Linseed oil meal.............	12	—	—	—	
Crude casein.................	10	20	20	20	—
Dried brewers' yeast.........	5	8	—	—	4
Lucerne meal.................	3	7	—	—	2
Butter fat...................	5	—	—		—
Beef liver (dried at 70° C.)....	2	—	—	—	—
Bone ash.....................	1	—	—		—
Cod liver oil................	1	5			5
$CaCO_3$.....................	0·5	1	—		1
NaCl.........................	0·5	—			—
Dextrinized starch...........	—	45	62·5	65	72
Lard.........................	—	10	15	15	10
Salt 40‡.....................	—	4		—	4
Agar.........................	—		2·5		2

* Plus tap water and fresh, whole milk *ad libitum*.

† The experimental stock ration used by French and Bloomfield to restore their depleted animals to normal weight was the same as that of Addis and co-workers (1926). The ration consists of maize starch 44, casein 16, lard 14, cod liver oil 10, salt mixture (Osborne and Mendel) 4, yeast 10, and lucerne meal 2 parts by weight. This ration seems to be complete in every respect and no doubt not inferior to the experimental stock ration used in this laboratory. Therefore, it is improbable that the difference in weight lost by rats during the first and second trials on the defective diet was due to the intermediate feeding by French and Bloomfield of a stock ration which was in itself not optimum in every respect.

‡ The composition of Salt 40 was similar to that of Steenbock and Nelson (1923) as modified by Keenan and others (1933).

Altogether there were six experiments. A description of these is given below and the data obtained on weight loss and food intake during these experiments are summarised in Table II. Furthermore, the average curves of each group for weight loss, food per rat per day,

* All the synthetic food mixtures were stored in a refrigerator, and, in order to prevent the development of rancidity, only enough of each ration to last for about three to four days was mixed at a time.

and food per 100 gm. rat per day, are given in figures I to VI. The solid lines are curves during the first trial on defective diet, the broken lines represent the second trial. In all the experiments the initial body weight is taken as 100 and the changes are expressed in actual grams lost per day.

Experiment I.

The 12 animals in this group were fed the vitamin and mineral low diet (but containing 2·5 per cent. agar) for 45 days when they had lost on the average about 21 per cent. of their body weight. They were then returned to the synthetic stock diet until they had regained their former weight and were then again fed the deficient diet for another 38 days. It is of interest to point out that seven days after the animals had been removed from the defective diet (after first trial with weight loss of 21 per cent.) and placed on the experimental stock diet, they weighed on the average 23 grams more than when they were first started on the deficient diet. In other words, the rats made an increase of 41·4 per cent. in body weight whereas during the week previous to the first trial on the defective diet they only gained 10·7 per cent. in body weight on the same diet. The indication of a stimulating after-effect of partial inanition on growth therefore supports the results of Kopec and Latyszewski (1932) obtained with mice. The remarkably quick recovery is also reflected by the food intake. During the seven days between the first and second trials on defective diet the animals consumed on the average 15·3 grams of the stock ration per rat per day or 7·5 grams per 100 gram rat per day whereas during the week previous to the first trial on defective diet they consumed 12·9 gm. of the stock ration per rat per day or 6·1 gm. per 100 gm. rat per day.

The curves in Fig. 1 show that the males lost less weight during the second trial on defective diet than during the first but also consumed slightly more food during the second trial than during the first. For the first 11 days the females also lost less in weight and consumed more food per rat per day during the second trial than during the first. After that time the curves crossed and the general picture was just the reverse. Because the difference in weight lost during first and second trials on defective diet seems to be a true reflection of the difference in food intake, it is difficult to resist the conclusion that, under the experimental conditions, the change in weight on defective diet was due primarily to the change in food intake.

Experiment II.

As the results obtained in experiment I do not support those of French and Bloomfield (1937), the experiment was repeated with 12 male and 12 female rats. These animals were fed the same deficient diet used in experiment I for 25 days each during the first and second trials.

From the curves given in Fig. 2, it is clear that the males again reacted as in the first experiment. The change in weight curve of the females is in agreement with those found by French and

Bloomfield in so far that the females lost appreciably more in weight during the first 12 days of the second trial than during the same period of the first. For 8 out of these 12 days the animals even consumed more food per rat daily during the second trial than during the first but, when expressed on the basis of a unit body weight (100 gm.), they actually consumed less during the second than during the first trial which may again help to account for the difference in weight lost.

Experiment III.

The deficient ration used in the two previous experiments differed from the ration used by French and Bloomfield only in so far as it also contained 2·5 per cent. of agar. Because of the difference between the results of these investigators and those of the writer, it was decided to omit the agar from the diet in order to see to what extent the agar was responsible for the discrepancy in results. The results obtained with 6 male and 6 female rats are depicted graphically in Fig. 3. The males again lost less weight during the second trial than during the first whereas there was hardly any difference between the weights lost by the females during the first and second periods on defective diet; and it is evident, therefore, that the agar was not the cause of the difference in results obtained in the two laboratories..

Experiment IV.

French and Bloomfield did not state whether their animals were kept on screens or not and it is possible, therefore, that their animals had access to their own excreta and that coprophagy took place. In order, therefore, to study the effects of a free access to excreta on the food intake and weight lost on a defective diet, 12 animals were kept on wood shavings in individual cages and fed the deficient diet used in experiment III. The curves given in Fig. 4 show again that the change in weight and food intake of the animals did not differ much during the first and second trials on the same diet.

Experiment V.

In view of the fact that, during the dry seasons the pasture in South Africa is of such a poor quality, the livestock, under ranch conditions, may not only suffer from a periodical vitamin (especially vitamin A) and a widespread mineral (Theiler and others, 1920, 1924. and du Toit and co-workers, 1932 and 1935) but also from a protein (Henrici, 1932a; 1932b, Henrici and Potter, 1934, and Smuts and others, 1939) shortage, a fourth group of 6 male and 6 female rats was included on a protein deficient ration. The ration was complete in every respect except that it was very low in proteins. It only contained about 0·34 per cent. nitrogen as supplied by the yeast and lucerne meal supplements. The first and second periods on this ration occupied 44 and 35 days respectively. The curves given in Fig. 5 show that, on the whole, there are no appreciable differences in the performance of the animals during the first and second trials on the low protein diet.

Experiment VI.

In order to make the experimental conditions as drastic as possible, a group of 12 (6 males and 6 females) adult rats were starved, except for distilled water, for 16 days each during the first and second trials. The curves given in Fig. 6 show that the animals lost weight at an equal rate during the first and second fasts.

SUMMARY.

(1) Data are presented on the change in body weight and food intake of young mature rats during the first and second trials on diets deficient in minerals and vitamins; minerals, vitamins and bulk; and proteins. Data are also given on the loss in body weight of rats during first and second fasts.

(2) The results show that, under the experimental conditions, rats which have lost weight as the result of a defective diet, and have then been restored to normal weight by stock ration did not show, as was found by French and Bloomfield, a more rapid weight loss, if now placed for a second time on the same defective diet. As a matter of fact in the majority of cases the animals lost slightly less weight during the second than during the first trial on defective diet. This was true no matter whether the rats had free access to their own excreta or not.

(3) Similarly, the food intake of the rats did not differ appreciably during successive periods on the same defective diet.

CONCLUSION.

There seems to be a positive relationship between the change in daily food consumption and the change in body weight. It is probable, therefore, that the reason why the animals of French and Bloomfield lost more rapidly in weight during the second than during the first trial on defective diet, was not because of a "latent deficiency" but because their animals consumed, on the whole, less food during the second than during the first trial on defective diet. Why that should have been so, is difficult to say, but it is possible that the palatability of their ration was not as good during the second as during the first trial.

REFERENCES.

ADDIS, T., MCKAY, E. M., and MCKAY, L. L. (1926). The effect on the kidney of the long continued administration of diets containing an excess of certain food elements. I. Excess of protein and cystine. *J. Biol. Chem.*, Vol. 71, pp. 139-156.

BLOOMFIELD, A. L. (1937). Individual variations in susceptibility to dietary deficiency, *J. Nutrition*, Vol. 14, pp. 111-116.

DU TOIT, P. J., MALAN, A. I., LOUW, J. G., HOLZAPFEL, C. R., and ROETS, G. C. S. (1932). A study of the mineral content and feeding value of natural pastures in the Union of South Africa (First Report). *18th Rep. Dir. Vet. Ser. and Anim. Indust.*, pp. 525-577.

DU TOIT, P. J., MALAN, A. I., LOUW, J. G., HOLZAPFEL, C. R., and ROETS, G. (1935). A study of the mineral content and feeding value of natural pastures in the Union of South Africa (Third Report), *Onderstepoort J. Vet. Sci. and Anim. Indust.*, Vol. 5, pp. 201-214.

FRENCH, L. R. and BLOOMFIELD, A. L. (1937). "Latent Deficiency" in rats: Variations in weight loss on repeated feeding of a defective diet, *J. Nutrition.* Vol. 14, pp. 117-129.

HENRICI, M. (1932). South African Pastures: Their mineral and protein content, *Farming in South Africa*, Vol. 7, pp. 245-248.

HENRICI, M. (1932). An investigation of the content of phosphorus, calcium and protein of grasses in the coastal region of Natal and Southern Zululand, *Sci. Bull.* 115, *Dept. Agric. of Union of South Africa*, 30pp.

HENRICI, M., and POTTER, P. E. (1934). An investigation of pastures in the Eastern Province and Albania Districts, *Sci. Bull.* 134, *Dept. Agric. of Union of South Africa*, 36 pp.

KEENAN, J. A., KLINE, O. L., ELVEHJEM, C. A., and HART, E. B. (1933). New nutritional factors required by the chick. *J. Biol. Chem.*, Vol. 103, pp. 671-685.

KOPEĆ, S., and LATYSZEWSKI, M. (1932). Untersuchungen über das Wachstum der Mäuse unter dem Einfluss intermittierender Fütterung. 1. Darreichung unvollwertiger Nahrung, Abwechselnd mit vollstandiger Karenz, *Biol. Generalis*, Vol. 8, pp. 163-178, *Biol. Abst.* (1934). Vol. 8, p. 1157.

SMUTS, D. B., MARAIS, J. S. C., and LOUW, J. G. (1939). The protein requirements of sheep on winter pasture in the Transvaal. *Farming in South Africa*, Vol. 14, pp. 153-155.

STEENBOCK, H., and NELSON, E. M. (1923). Light in its relation to ophthalmia and growth, *J. Biol. Chem.*, Vol. 56, pp. 355-373

THEILER, A., VILJOEN, P. R., GREEN, H. H., DU TOIT, P. J., and MEIER, H. (1920). The cause and prevention of lamziekte, *J. Dept. Agric., South Africa*, Vol. 1, pp. 221-224.

THEILER, A., GREEN, H. H., and DU TOIT, P. J. (1924). Phosphorus in the live stock industry, *J. Dept. Agric., South Africa*, Vol. 8, pp. 460-504.

EXPERIMENT I.

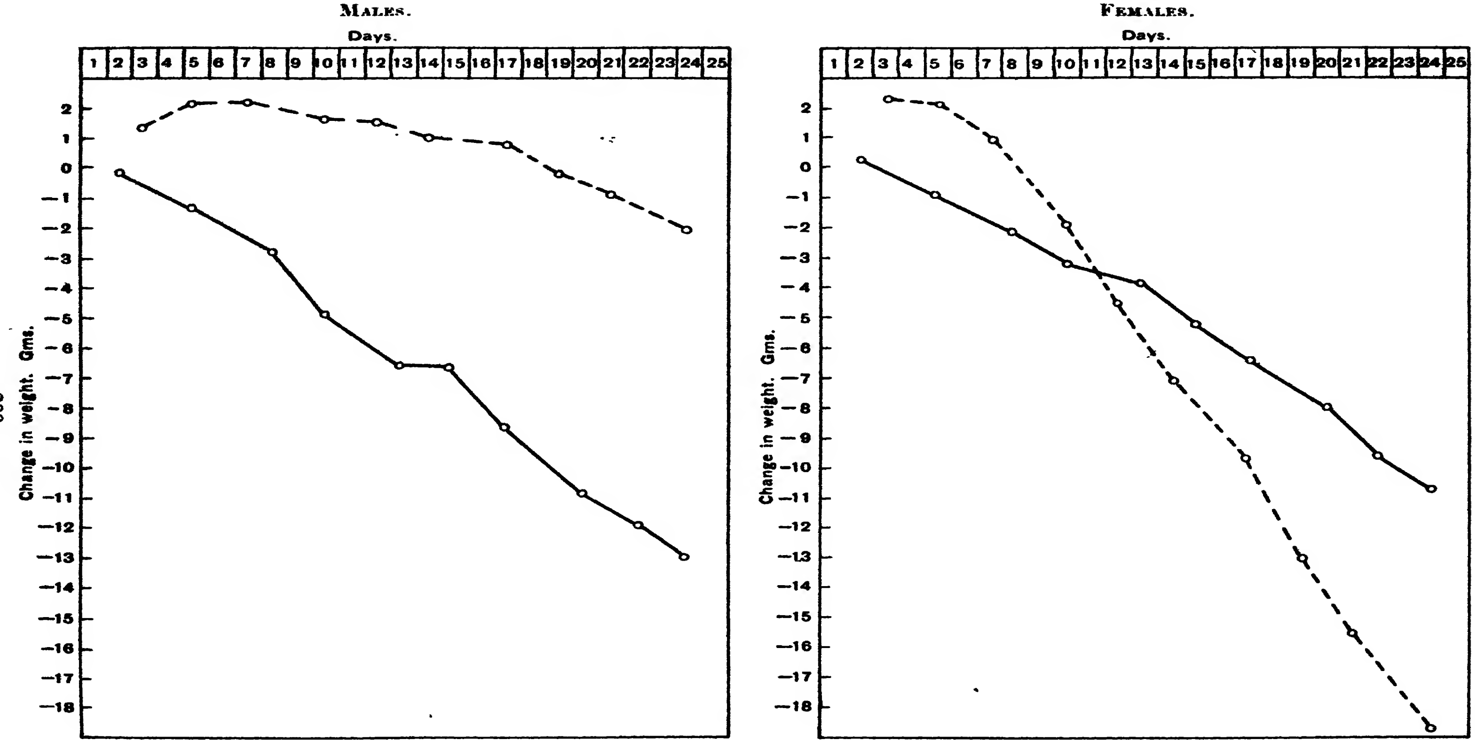
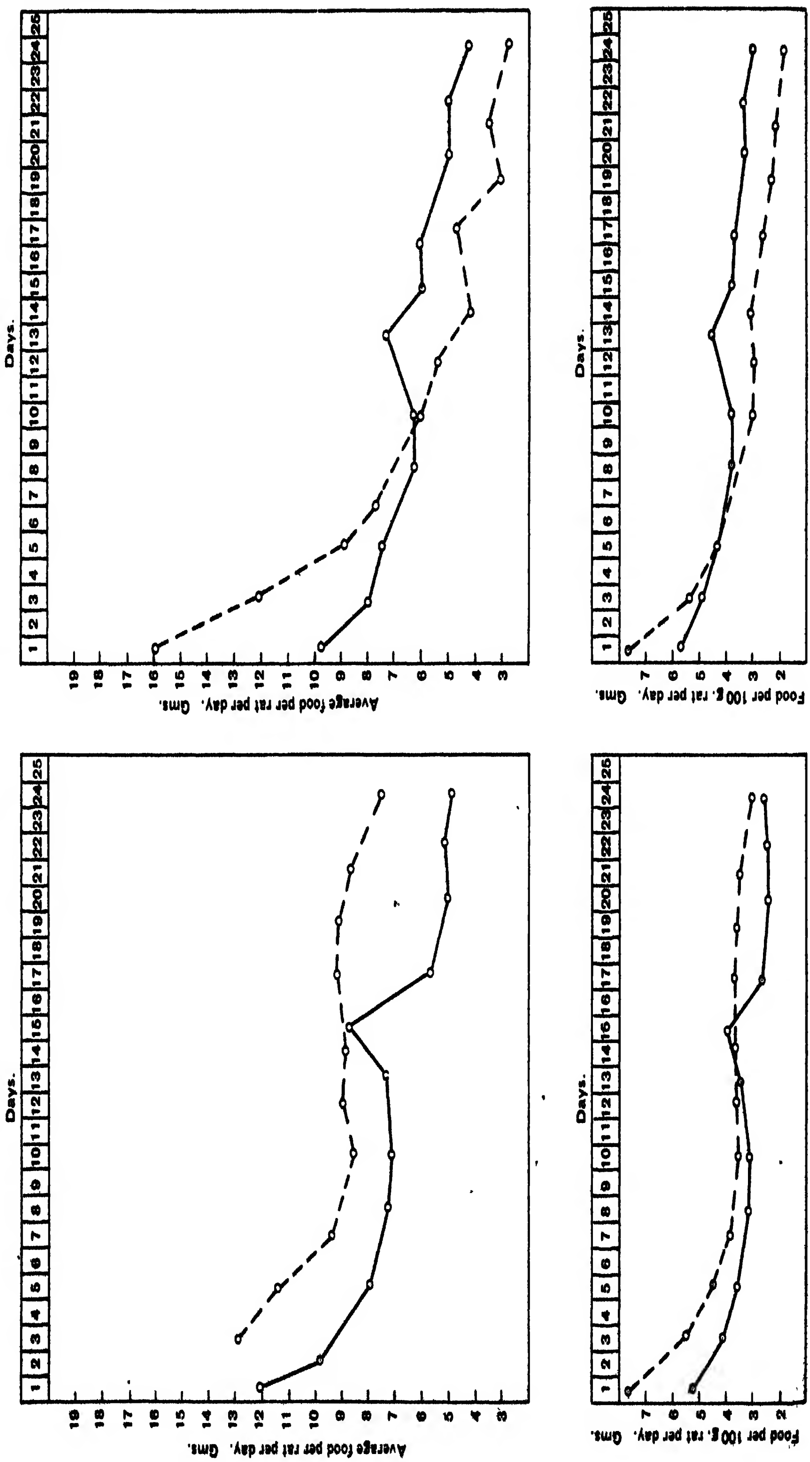


Fig. 1.—Graph of data from Experiment I (see text). The solid lines are curves during first trial on defective diet, the broken lines represent the second trial.

EXPERIMENT I (*continued*).

Fig. 1 (*continued*).



EXPERIMENT II.

MALES. FEMALES.

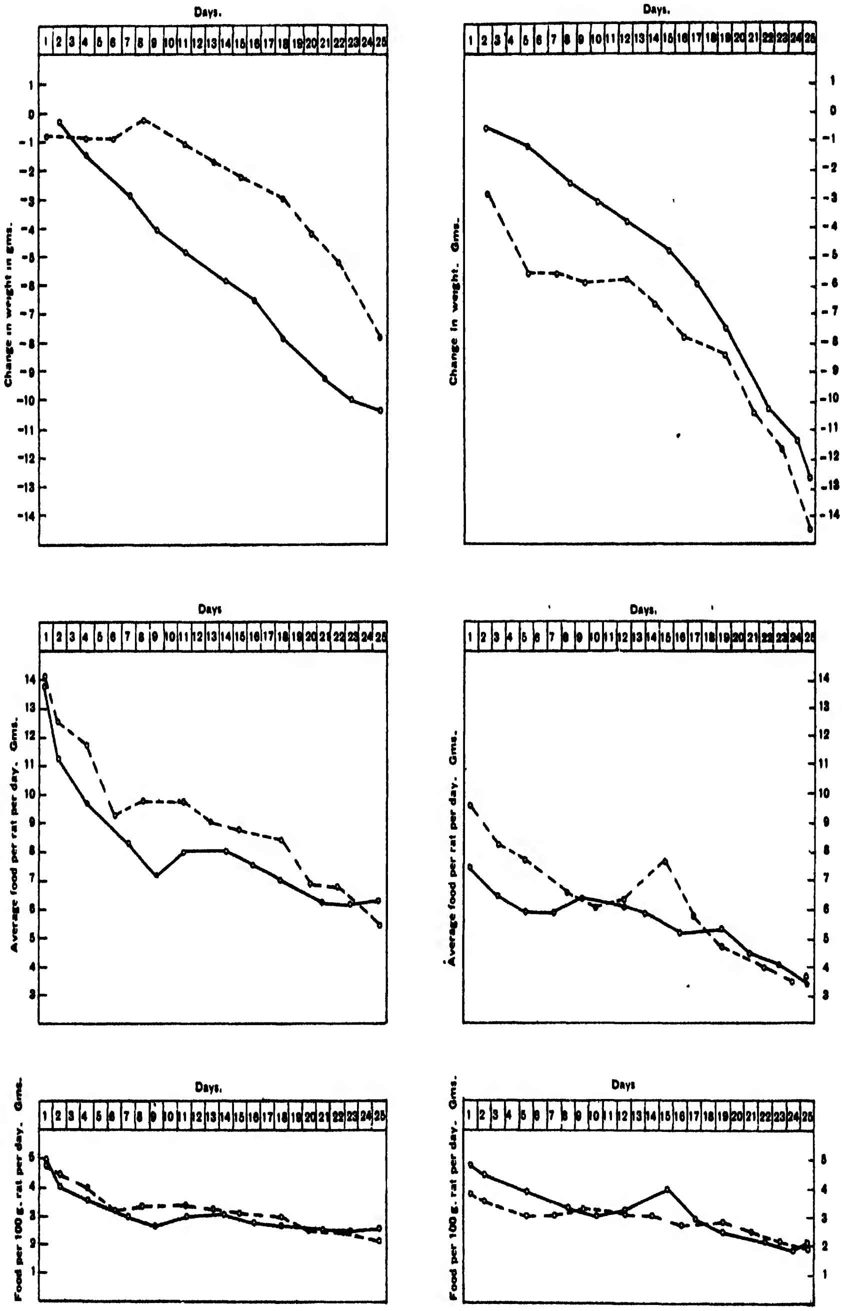


Fig. 2.—Graph of data from Experiment II (see text). The solid lines are curves during first trial on defective diet, the broken lines represent the second trial.

EXPERIMENT III.

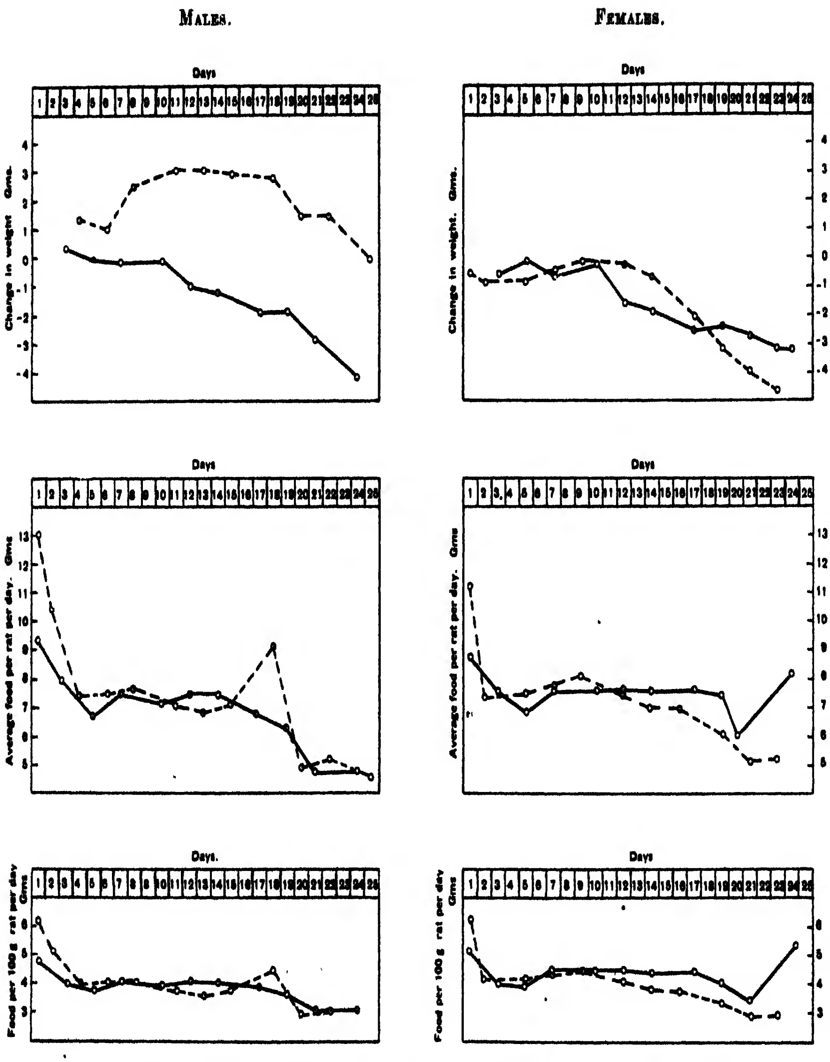


Fig. 3.—Graph of data from Experiment III (see text). The solid lines are curves during first trial on defective diet, the broken lines represent the second trial.

EXPERIMENT IV.

MALES. **FEMALES.**

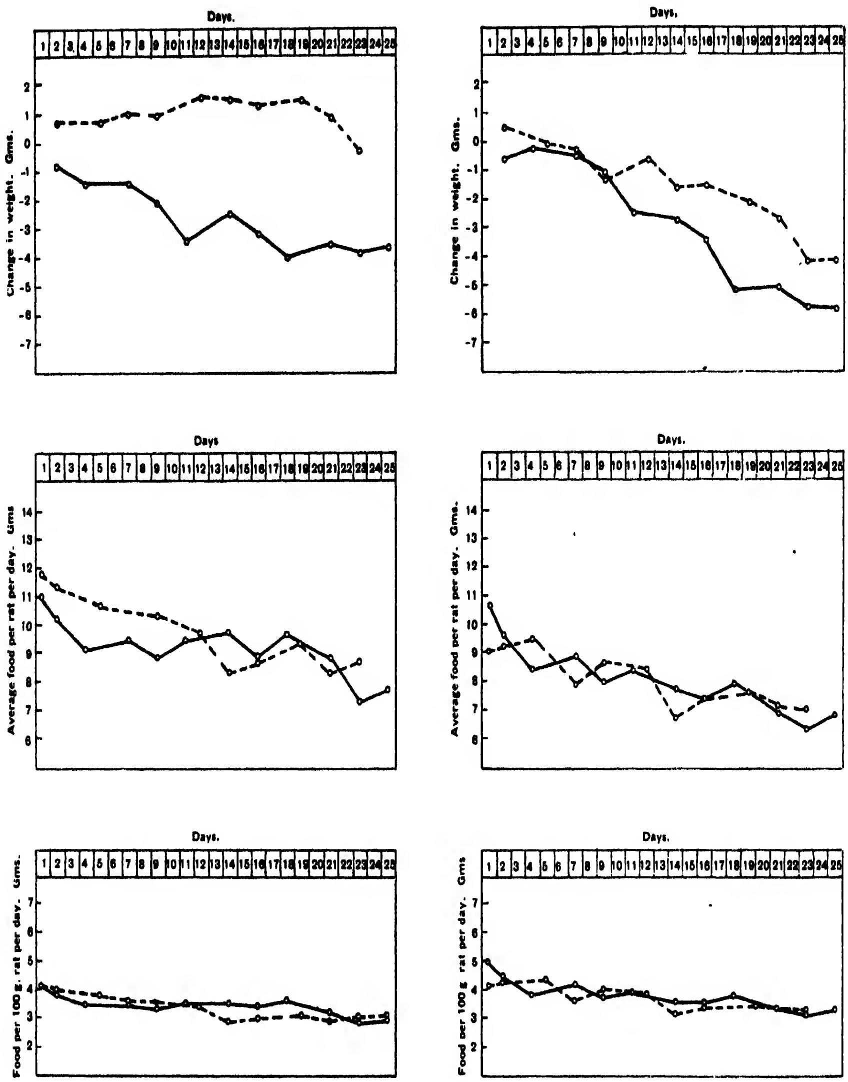


Fig. 4.—Graph of data from Experiment IV (see text). The solid lines are curves during first trial on defective diet, the broken lines represent the second trial.

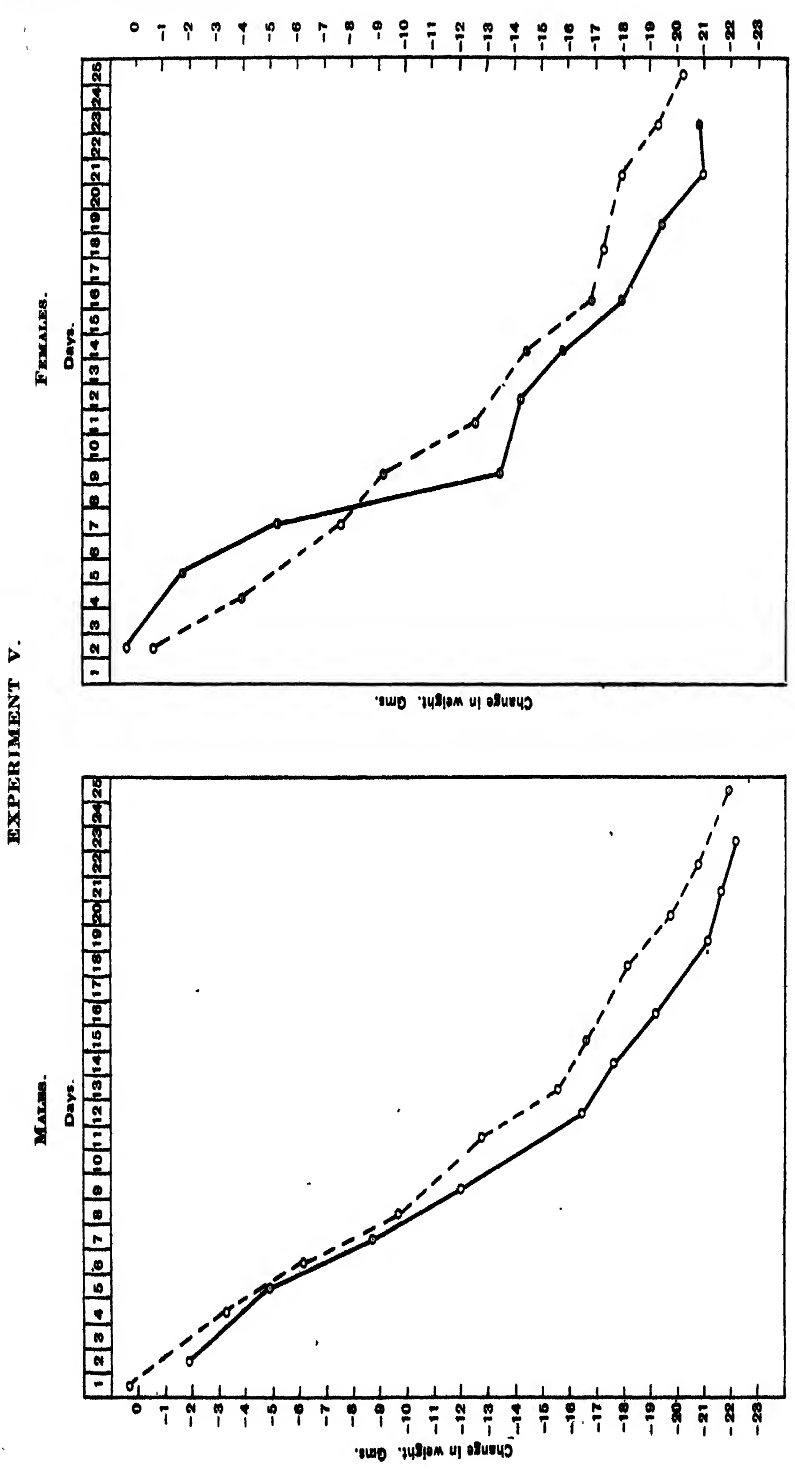
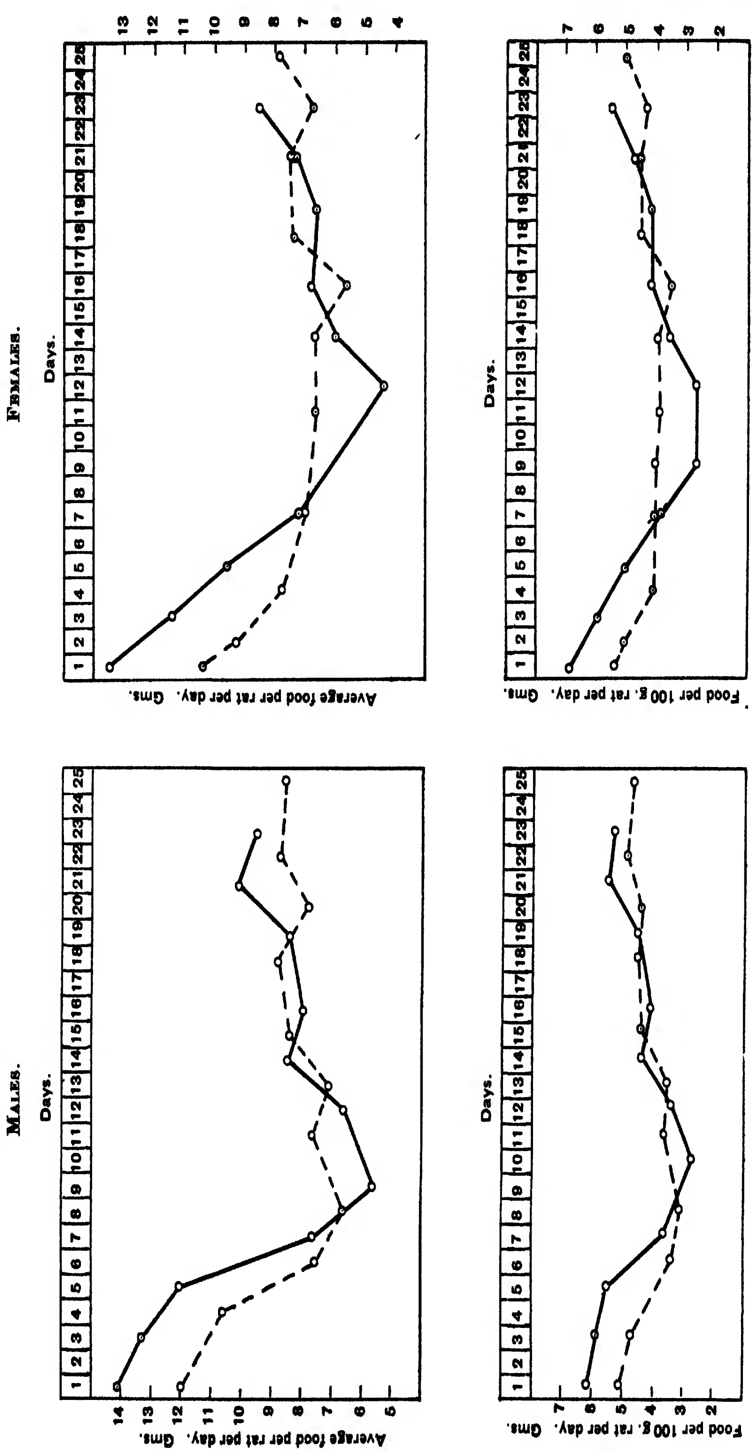


Fig. 5.—Graph of data from Experiment V (see text). The solid lines are curves during first trial on defective diet, the broken lines represent the second trial.

EXPERIMENT V (continued).

Fig. 5 (continued).



EXPERIMENT VI.

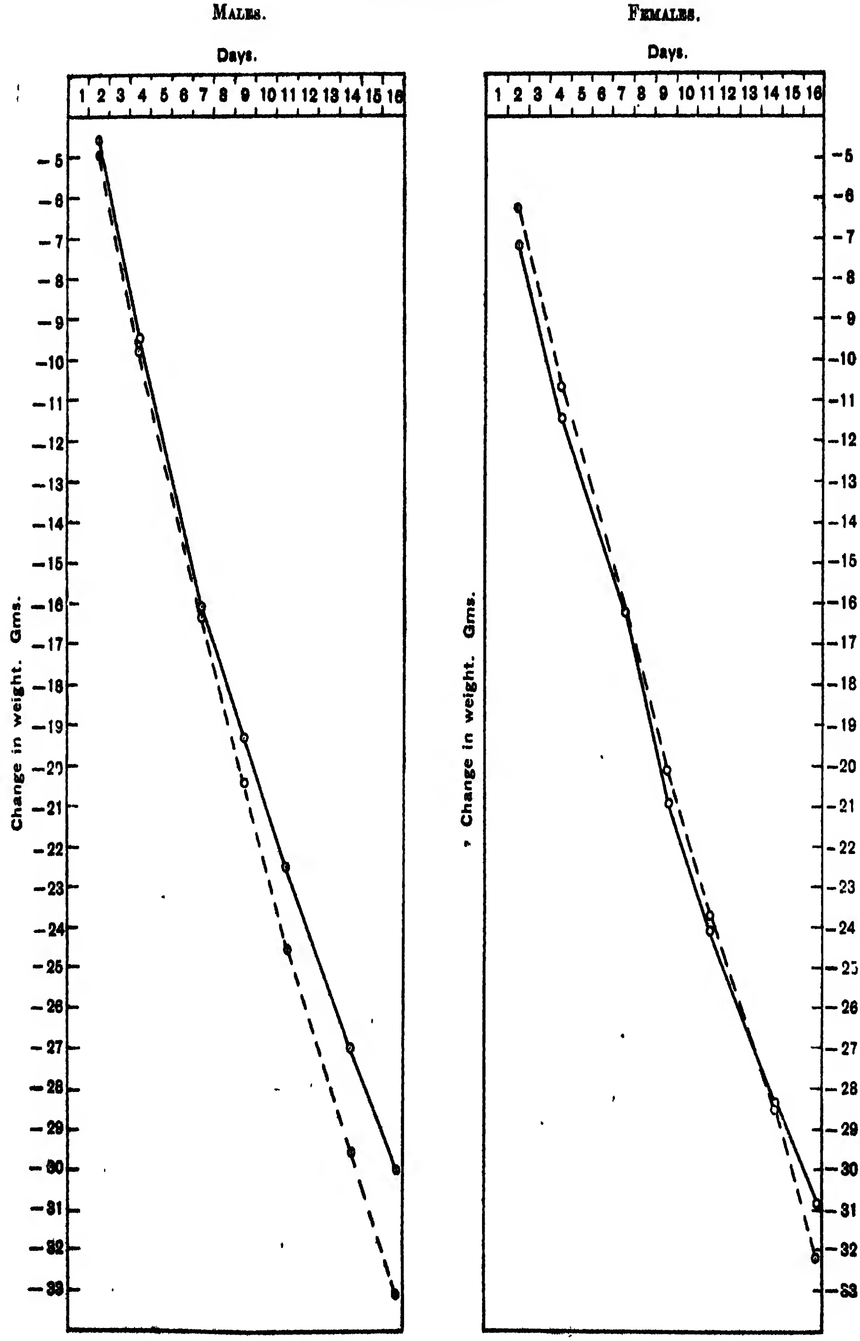


Fig. 6.—Graph of data from Experiment VI (see text). The solid lines are weight curves during the first fast, the broken lines represent the second fast.

Section VI.

Nutrition.

SMUTS, D. B. AND MARAIS, J. S. C.	The endogenous nitrogen metabolism of young sheep with reference to the estimation of the maintenance requirement of sheep.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 13, Number 1, July, 1939.

Printed in the Union of South Africa by the Government Printer, Pretoria.

The Endogenous Nitrogen Metabolism of Young Sheep with Reference to the Estimation of the Maintenance Requirement of Sheep.

By

D. B. SMUTS, and J. S. C. MARAIS, Section of Nutrition, Onderstepoort.

That the maintenance requirement of nitrogen of an animal can be measured by the total nitrogen excretion in the urine after the endogenous level is attained, is now generally accepted. On this basis, the endogenous nitrogen excretion of mature sheep was measured (Smuts and Marais 1938) and the results interpreted in relation to the maintenance requirement of protein. However, it is evident from the literature that the relationship between the endogenous nitrogen and basal metabolism established by Smuts (1935) for mature animals may also hold good for young animals. Du Bois (1916) with human beings, Deighton (1934) with pigs, Mitchell (1926) with rats, and Ritzman and Benedict (1930) with sheep, have shown that the basal metabolism of immature animals is invariably higher than that of mature animals of the same species. On the other hand, from data of Terroine (1933) with rats, it appears that the endogenous nitrogen metabolism reacts in the same way and follows the same general trend as the basal metabolism. Consequently it follows that the endogenous nitrogen metabolism, and therefore the maintenance requirement of protein for immature sheep will be greater than that already established by us for mature sheep.

In this study an effort was made to measure the endogenous nitrogen metabolism of young sheep with a view to establishing their maintenance requirement.

Experimental.

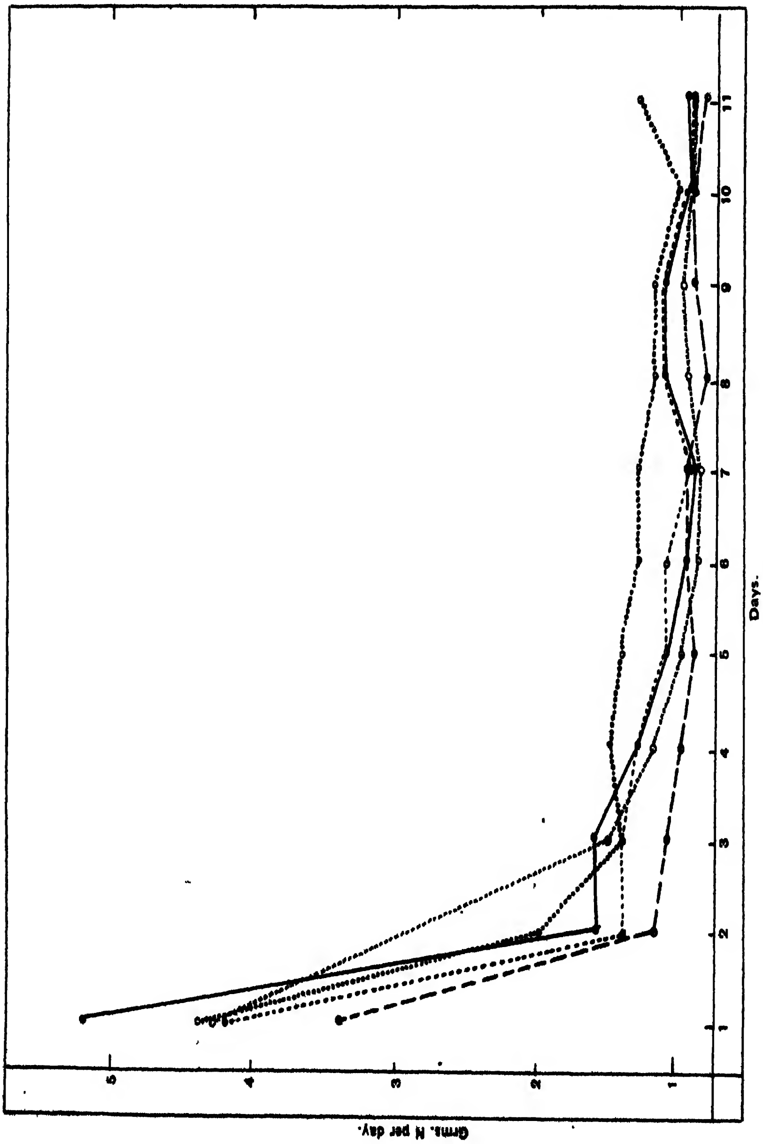
Nine young Merino wethers approximately four months of age, and weighing from 17 to 28 Kgms., were utilized. These sheep were put for 3 weeks on a standard ration containing 14 per cent. lucerne protein. After the termination of this preliminary period, they were removed to the metabolism cages and put on a nitrogen-low ration, the composition of which is the same as previously described by Smuts and Marais (1938). It was, however, found

necessary to include 10 per cent. of wheat straw and to reduce the agar to 15 per cent. in order to ensure a high enough food intake to cover the energy requirements. By this method it was possible to study the complete curve of nitrogen excretion.

Experimental Results.

Curves representing the total nitrogen excretion in the urine of the 9 sheep on a nitrogen low ration are given in graphs 1 and 2. In comparison with mature sheep kept under the same standard

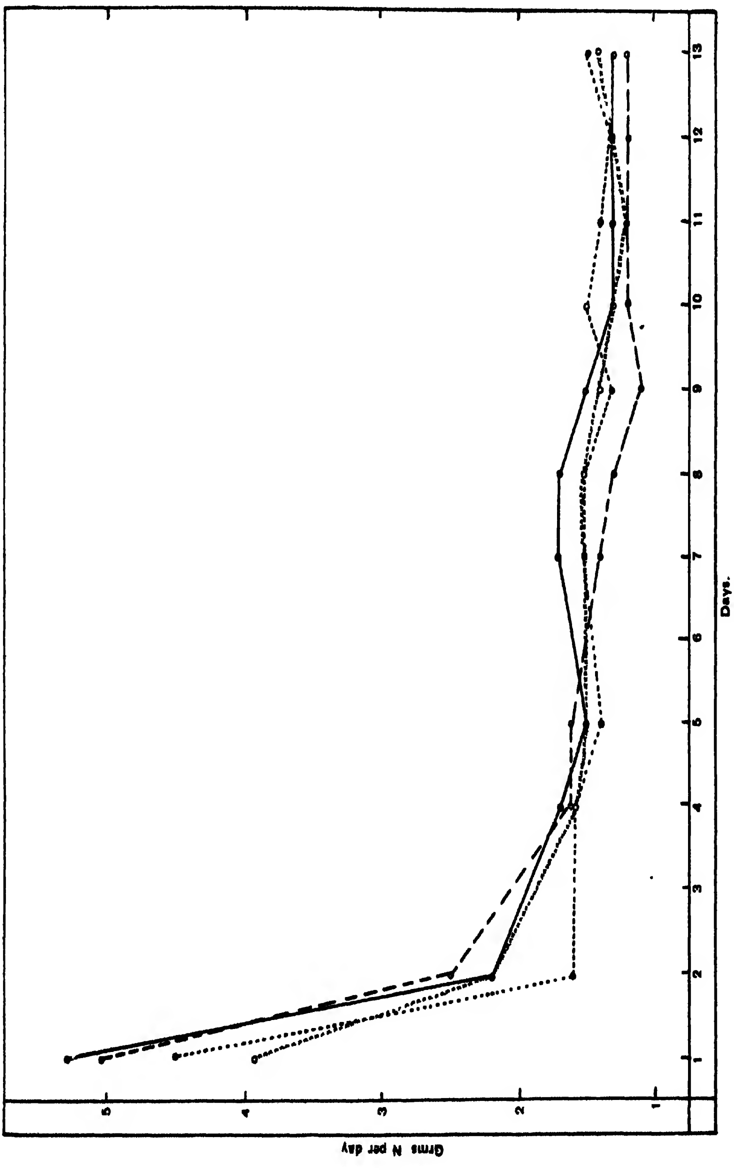
Graph 1.
Endogenous Nitrogen Excretion of Sheep on a N-low ration.



conditions of feeding, namely 14 per cent. lucerne protein, there exists a distinct difference in the nitrogen elimination, when placed on a nitrogen low ration. With mature sheep, it was noticed that after the initial sharp drop in nitrogen excretion on the first day, there was a further prominent decrease up to the eighth day. Thereafter the decrease was more gradual, and such that the

GRAPH 2.

Endogenous Nitrogen Excretion of Sheep on a N-low ration.



endogenous level was only reached round about the 14th day. With young sheep, as will be seen from the graphs, the position is very different. There is in each case the characteristic sharp drop in the nitrogen excretion the first day, with a very gradual reduction up to the 5th or 6th day, when in nearly every case the endogenous level is attained. From there onwards the curve representing the daily urinary nitrogen almost assumes a straight line representing a very constant output. Thus there appears to be a distinct difference in the amount of nitrogen stored by the actively growing sheep and the non-growing or mature sheep. Such a difference may be expected on the basis that the growing animal needs in addition to its maintenance requirement, a substantial portion of the available nitrogen for tissue synthesis, leaving thus a much smaller fraction of the total nitrogen intake for reservation. This statement is endorsed by the fact that mature sheep on an 8 per cent. lucerne protein ration for 3 months, attain their endogenous level after 6 days on a nitrogen low ration, in comparison with 14 days after a 14 per cent. lucerne protein ration. This indicates, that at the higher level of protein feeding almost twice as much nitrogen was available for storage as at the lower level.

The endogenous nitrogen per unit weight in accordance with the general view, is higher for young sheep than for mature ones. The average figure recorded by us for mature sheep is ·041 grms. per Kg. weight, while for immature sheep the average figure obtained in this study is ·051 grms. per Kg. Expressed on the basis of utilizable protein these figures per 100 lb. weight become 11·6 grms. and 14·4 grms. protein.

In trying to find a general method for estimating the protein requirements for maintenance, it was stated in an earlier paper [Smuts and Marais (1938)] that the formula adopted by Smuts (1935) for the prediction of the protein requirements of mature animals of different species could be applied to sheep. However, after assembling all the data thus far obtained on the endogenous nitrogen excretion of sheep, it was found that the percentage deviation from the determined values by the application of the above formula could be decreased considerably by the introduction of a different constant. In Table 1 the data pertaining to the endogenous nitrogen determinations are tabulated. From these figures a value for k in the formula $P = kW^{\cdot 734}$ has been established. As will be seen, the same value for k was obtained for mature and young sheep. This value was then introduced into the formula and the utilizable protein predicted, as shown in column 6. In column 7 the percentage deviation has been determined. For mature sheep this deviation is only $\pm 2{\cdot}5$ per cent., and with young sheep $\pm 5{\cdot}4$ per cent. The magnitude of these deviations is considered exceptionally small and much less than the deviation when the general formula $P = {\cdot}88\ W^{\cdot 734}$ for different species is applied. Consequently the new formula $P = {\cdot}74\ W^{\cdot 734}$ appears to be better applicable for the estimation of the protein requirements for maintenance of mature as well as young sheep. In this formula P equals utilizable protein and W weight in Kgm.

TABLE 1.

Formula for Predicting the Endogenous Nitrogen (Protein) of Sheep.

Animal No.	Weight in Kgm.	End. N. as Determined.	End. N. Expressed as Protein.	Value k in $P = kW^{\cdot 734}$	End Protein $P = \cdot 74\ W^{\cdot 734}$	Percentage Deviation from Value.
			MATURE SHEEP.			
1	44·5	2·03	12·69	·78	11·99	− 5·5
2	40·0	1·81	11·34	·75	11·09	− 2·2
3	37·7	1·72	10·75	·75	10·62	− 1·2
4	42·0	1·84	11·50	·74	11·50	0·0
5	39·0	1·72	10·75	·73	10·89	+ 1·3
7	40·0	1·76	11·00	·73	11·09	− 0·8
8	44·0	1·79	11·19	·70	11·90	+ 6·4
9	50·0	2·01	12·56	·72	13·07	+ 4·06
10	40·0	1·75	10·95	·73	11·09	+ 1·3
		Average...	—	·74	—	± 2·5
			YOUNG SHEEP.			
1A	17	·907	5·67	·71	5·92	+ 4·4
2A	20	1·02	6·38	·70	6·67	+ 4·5
3A	22	1·00	6·25	·65	7·15	+14·4
4A	18	·930	5·81	·70	6·17	+ 6·2
5A	23	1·20	7·48	·75	7·39	− 1·2
6A	22	1·23	7·69	·80	7·15	− 7·0
8A	26	1·34	8·38	·77	8·09	− 3·5
9A	25	1·33	8·34	·79	7·86	− 5·4
10A	28	1·40	8·75	·76	8·54	− 2·4
		Average...	—	·74	—	± 5·4
		Average of Total........		·74	—	± 4·4

In Table 2 the utilizable protein, digestible protein, and the minimum energy requirements (basal metabolism), have been predicted. The utilizable protein is directly calculated by means of the above equation, while the digestible protein is calculated from

TABLE 2.

Prediction of Maintenance Requirement for Protein for Sheep.

Weight in Kgm.	Weight in lb.	Utilizable Protein ($P = 74W^{\cdot 734}$)	Digestible Protein.	Basal Metabolism. Cal/day.	Basal Metabolism Cal/per Kgm.
15	33	5·40	10·80	432	29
20	44	6·67	13·34	534	27
30	66	8·94	17·88	715	24
35	77	10·06	20·12	805	23
40	88	11·09	22·18	887	22
45	100	12·10	24·20	968	22
50	110	13·07	26·14	1,046	21

the utilizable protein by assuming a biological value of 50. The latter assumption naturally provides for a fair margin of safety, since at maintenance level we have not experienced a biological value as low as 50.

The basal metabolism is calculated by assuming that the relationship of 2 mgm. endogenous nitrogen or 12·5 mgm. protein is equivalent to one Calorie of basal heat.

It is interesting to note that the digestible protein requirement as predicted by the general equation of $P = \cdot 88\ W^{\cdot 731}$ for a 100 lb. sheep is 29 grams, by the new equation 24 grams, and according to Armsby (1917) 27 grams. Naturally Armsby's figure holds good for any ration, while the figure obtained in this study will be greater or smaller depending on the biological value of the feed.

The basal metabolism in column 5 and expressed per Kg. weight in column 6 is interesting in view of the fact that it allows for an easy means of predicting the minimum energy requirement. A 100 lb. sheep according to our calculations would have a basal metabolism of 22 Calories per Kg. weight. This figure coincides with the average value of the W-W wethers, W 2-5, W2-41, W 2-12 and W 2-6 of Lines and Pierce (1931). Their average value for the four sheep weighing from 41 to 52 Kgms. is 22 calories per Kgm. Ritzman and Benedict (1930) obtained an average value of 27 calories per Kg. which is slightly higher than our figure. These sheep, however, were measured for their basal metabolism 18 to 37 hours after withdrawal of food and may probably not have reached the post absorptive condition. Armsby (1917) on the other hand, basing his calculations on the work of Henneberg and Kellner, arrived at a value of 16 calories per Kg.

For the EE lambs of 5 months of age, Lines and Pierce (1931) obtained an average value of 36 calories per Kg. This value is higher than our predicted value of 29 calories. However, it appears from the work of the above authors that the nutritional level of the animal or the seasonal changes in pasture may effect the basal metabolism. The seasonal effect is claimed to be due to the change in protein content. These factors may therefore partly be responsible for our lower endogenous nitrogen and consequently a lower predicted figure for the basal metabolism, since our sheep were kept on a low level of protein for a considerable time.

From the basal metabolism figures of Lines and Pierce (1931), and Ritzman and Benedict (1930), and our calculated values from the endogenous nitrogen, it appears that a very definite relationship exists between the basal metabolism and the endogenous nitrogen metabolism in sheep. Such a relationship would in future be of considerable value in assessing the value of either of these entities once the magnitude of one is known.

Summary and Conclusions.

In a study on the endogenous nitrogen excretion of immature sheep, it was found that young sheep reach their endogenous level on the 6th day, after having been on a standard ration of 14 per cent. lucerne protein. The endogenous nitrogen excretion was found

to be higher than in mature sheep, the average value for 4 months old wethers being ·051 grms. per Kg. A formula for estimating the maintenance requirement of sheep was devised and its application tested. It was also shown that the basal metabolism of sheep can be predicted from the endogenous N, and that the figures arrived at agree very well with the values reported in the literatuie.

LITERATURE.

ARMSBY, H. P. (1917). The nutrition of farm animals, p. 326. New York.

DEIGHTON, T. (1934). A study of the fasting metabolism of various breeds of hog. 1. Age, weight and length data *J. Agric. Science*, Vol. 24, pp. 326-334.

DU BOIS, E. F. (1916). The respiration calorimeter in clinical medicine, *Am. J. Med. Sci.*, Vol. 151, pp. 781-799.

MITCHELL, H. H. and CARMAN, G. G. (1926). Effect of excessive amounts of vitamin B on the basal metabolism of rats of different ages, *Amer. J. Physiol.*, Vol. 76, pp. 385-397.

LINES, E. W. and PIERCE, A. W. (1931). The basal (standard) metabolism of Australian Merino Sheep. *Com. of Australia. Counc. Sci. and Ind. Research*, Bull. No. 55, p. 33.

RITZMAN, E. G. and BENEDICT, F. G. (1930). The energy metabolism of sheep. *Univ. of New. Hampshire. Exp. St.*, Bull. 43, p. 23.

SMUTS, D. B. (1935). The relation between the basal metabolism and the endogenous nitrogen metabolism, with particular reference to the *Nutrition*, Vol. 9, pp. 403-433.

SMUTS, D. B. and MARAIS, J. S. C. (1938). The endogenous nitrogen metabolism of sheep with special reference to the maintenance requirements of protein, *Onderstepoort Jour. Vet. Sci. and An. Ind.*, Vol. 11, No. 1, pp. 131-140.

TERROINE, E. F., BOY, G., CHAMPAGNE, M., MOURAT, G. (1933). La répartition des représentants Urinaires du métabolisme Azoté et a signification physiologique II. La dépense Azoteéendogené spécifique minicura du coms de la Croissance. *Bul. Soc. Chem. Biol. T.*, 15, p. 42.

Section VII.

Toxicology

AND

Poisonous Plants.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 13, *Number* 1, *July*, 1939.

Printed in the Union of South Africa by the Government Printer, Pretoria.

Chemical Investigations upon *Lotononis laxa* E. and Z.I. The Isolation of Pinitol, a Fatty Ester and Benzaldehyde.

By H. L. DE WAAL, Section of Pharmacology and Toxicology, Onderstepoort.

This plant was responsible for prussic-acid poisoning in stock on cultivated lands in the Lady Grey district, C.P., and was submitted to this laboratory for a chemical examination of the toxic principle. The dried and ground plant-material, after it had been for approximately six weeks at the laboratory still contained 259·2 mgm. HCN per 100 grams of dried plant (i.e. about 0·26 per cent. HCN), which obviously is a very high figure, making the plant extremely dangerous to stock.

Determination of Hydrocyanic-acid Content.

The hydrocyanic-acid determinations were carried out in the usual way with samples of 10 gms. of the dried and ground plant with the following results:—

Time of Maceration.	Medium.	Enzyme.	Temperature.	c.c. $\frac{N}{50}$ $AgNO_3$.
4 hours	200 c.c. dist. H_2O...............	Plant only...	Room.....	12·1
6 hours	,, ,, ...	,,	,,	14·0
16½ hours	,, ,, ...	,,	,,	21·0
18½ hours	,, ,, ...	,,	,,	21·0
17 hours	200 c.c. pH6 (buffer solution)....	,,	,,	24·0*
19 hours	,, ,, ,, ...	,,	,,	24·3*
24 hours	,, ,, ,, ...	,,	,,	24·5*
25 hours	,, ,, ,, ...	,,	,,	24·3*
20 hours	,, ,, ,, ...	Plant enzyme and emulsin	,,	23·5

* The maximum HCN content was obtained after about 18 hours maceration in a buffer solution of pH6 at room temperature (plant enzyme only).

Cyanhydrin-test.—10 gms. of the dried powdered plant was macerated with 200 c.c. of a buffer solution of pH = 6 for 20 hours (no emulsin). 50 c.c. Normal caustic soda was then added (distinctly

alkaline) and allowed to stand for 20 minutes (for hydrolysis of possible cyanhydrins). 20 gm. Tartaric acid was then added (distinctly acid) and the HCN distilled. On titration of the clear distillate 23·5 c.c. $\frac{N}{50}$ $AgNO_3$ was used.

Therefore neither the addition of emulsin nor the hydrolysis of possible cyanhydrins showed an increase in the total hydrocyanic acid content.

$\therefore$ The maximum HCN content = 24·0 × 1·08 × 10.
= 259·2 mgm. HCN per 100 gms. dry plant.

In each case the distillate was collected in $\frac{N}{3}$ NaOH and had a strong aromatic odour, similar to that of bitter-almonds, and when the distillate was acidified to about 2 N-HCl and treated with a solution of Brady's reagent (2, 4-dinitrophenylhydrazone) in 2 N-HCl solution a strong precipitate was observed (see below for nature of precipitate). The aromatic substance had aldehydic properties.

Detection and Determination of Benzaldehyde.

When the dried and powdered plant was immediately steam distilled in either (1) a neutral solution, i.e. suspended in distilled water or (2) in an alkaline medium (i.e. about 20 gms. plant in 50 c.c. normal NaOH) distillates were obtained, which smelt strongly aromatic (bitter-almonds) and which gave strong orange-yellow precipitates with Brady's reagent.

The following experiment was conducted to determine the yield of the Brady's derivate:—

(I) 50 gm. of dried and ground plant-material (HCN content = 0·259 per cent.) was suspended in 200 c.c. of a citrate buffer solution (pH = 6) and immediately steam distilled. The distillate was collected in a little ice-water. Hydrocyanic acid was freely liberated. The distillation was stopped after 50 minutes, which proved sufficient for the complete recovery of the aromatic substance.

The distillate (about 180 c.c.) was acidified with concentrated hydrochloric acid to about 2-normal and warmed. To the warm solution a slight excess of hot Brady's reagent (0·5 gm. in 15 c.c. 2 N-HCl) was added. An orange-yellow precipitate formed immediately which was centrifuged off after some time. The precipitate was thoroughly washed first with 2 N HCl and then with distilled water and finally dried to a constant weight at 110° C. Weight of precipitate = 0·2 gm.

The crystalline precipitate was easily recrystallized from either acetone or alcohol and after two re-crystallizations from either solvent had a constant, clear melting-point of 235° C., formed fine

orange leaflets and was chemically pure. The crystals dissolved fairly easily in acetone and ethylacetate, dissolved with difficulty in hot absolute alcohol and were insoluble in water.

*Micro-analysis.**

5·191 mgm.	10·400 mgm. CO_2; 1,720 mgm. H_2O.
3·373 mgm.	0·562 c.c. N at 20·5° C. and 765 mm. Hg.
Found	C = 54·66%; H = 3·71%; N = 19·50%.
Calculated for $C_{13}H_{10}N_4O_4$	C = 54·56%; H = 3·52%; N = 19·57%.

i.e. Benzaldehyde—2, 4-dinitrophenylhydrazone or $C_6H_5\underset{H}{C} = N - \underset{H}{N} - C_6H_3(NO_2)_2$

* All micro-analyses by Dr. Ing. A. Schoeller, Berlin.

When this substance was mixed with an authentic specimen of benzaldehyde - 2, 4-dinitrophenylhydrazone prepared by the condensation of the components in HCl medium, no depression of the melting-point occurred. The authentic sample crystallized from acetone or alcohol and also had m.p. 235.

(II) The above steam disillation (I) was repeated and the distillate collected in ice-water. The distillate was then shaken with pure ether (Merck), the ethereal solution washed, dried over Na_2SO_4, filtered and allowed to evaporate at room temperature. A little absolute alcohol was then added to the residue, which smelt strongly of benzaldehdye, and the solution refluxed with 0·3 gm. semi-carbazide-hydrochloride for 30 minutes. The filtrate was then slightly evaporated and the micro-crystalline material re-crystallized from very dilute alcohol. Colourless slender needles separated which had a melting point of 214° C. This is also the m.p. of benzaldehyde-semi-carbazone.

An authentic specimen was therefore prepared from benzaldehyde (Merck) and semi-carbazide-hydrochloride. Crystallized from alcohol, the synthetic benzaldehyde-semi-carbazone (shining plates) had a m.p. of 214° C. The natural and synthetic specimens were mixed, and the m.p. of the mixture showed no depression.

The substance was therefore benzaldehyde-semi-carbazone.

As can be seen from the above the yield of benzaldehyde was very small. When the steam-distilled plant was therefore again macerated with emulsin in a buffer solution of pH = 6, the major portion of the hydrocyanic acid could be determined. It is thus clear that the major portion of the hydrocyanic-acid is present in the form of a substance capable of being hydrolised by an enzyme.

The Nature of the Cyanogenetic Glucoside.

With the small and inadequate quantity of plant material at our disposal the preliminary attempts to isolate the cyanogenetic glucoside failed. Only benzaldehyde, pinitol and a fatty ester could be isolated thus far and the isolation of the cyanogenetic constituent is reserved for a later date when more plant material will be available.

Preliminary results however would point to the possibility that the nature of the cyanogenetic glucoside may be that of a combination of benzaldehyde and hydrocyanic acid, e.g. with glucose or vicianose. Such examples are amygdalin, sambunigrin (see also Finnemore et al), vicianine (Bertrand), Prunasine (Fischer and Bergmann) and prulaurasine (Fischer and Bergmann). These glucosides are all highly toxic due to their ready hydrolysis to benzaldehyde, hydrocyanic acid and the sugar constituent.

Isolation of a Fatty Ester, Probably $C_{42}\ H_{84}\ O_4$.

When separate quantities (about 100 gm.) of the dried and ground plant were extracted in a Soxhlet apparatus with (*a*) ether, (*b*) acetone, (*c*) petroleum-ether and (*d*) ethyl-acetate, a fatty-like crystalline powder was obtained in each case. After re-crystallization from acetone the crystalline powder had a melting-point of 78° C. (clear).

The substance was insoluble in water, alkalis, mineral acids and dilute sodium carbonate solution. It was very soluble in chloroform, and soluble in absolute alcohol, ethyl-acetate and acetone. It was difficultly soluble in ether and petroleum-ether.

The substance gave no colouration with concentrated sulphuric-acid or with ferric-chloride solution. Phytosterol tests were negative. It is a neutral, non-acidic, non-phenolic, optically inactive substance and contained carbon, hydrogen and oxygen only.

Micro-analysis.

4·936 mgm....................	14·680 mgm. CO_2; 5·950 mgm. H_2O.
Found........................	C = 81·15%; H = 13·49%.
Calculated for $(C_{21}H_{42}O)x$......	C = 81·30%; H = 13·55%.

Molecular weight determinations in camphor (Rast) gave results for a molecular weight of 800 to 1200. Now $C_{42}H_{84}O_2$ (molecular weight 620) is the cerylester of palmitic acid, the main constituent of opium wax; has a melting-point of 79° C. and is a crystalline powder (Beilstein, Heilbron).

It has the formula $CH_3.(CH_2)_{13}.CH_2.CO\text{-}O\text{-}CH_2.(CH_2)_{24}.CH_3$, which may be the same as the above substance isolated from *Lotononis laxa* E and Z.

Isolation of Pinitol.

The dried and powdered plant was extracted with acetone in a Soxhlet apparatus. When the neutral substance of m.p. 78° C. (see above) had separated out the acetone solution was filtered and decolourised with adsorbent charcoal. After filtration an aliquot was diluted with about an equal volume of benzene when crystals of m.p. 181-183° separated out. When the colourless acetone solution was allowed to stand in an ice-chest the same substance separated together with a syrupy liquid, which was positive for sugar and slightly positive for cyanogenetic glucoside.

However, very little cyanogenetic glucoside must have been extracted since the plant-residue still contained about 92 per cent. of the original hydrocyanic acid content. Neither did digestion with cold acetone, nor Soxhlet extraction with ether or petroleum-ether remove any of the cyanogenetic glucoside. Hot acetone and hot ethyl-acetate extracted some of the cyanogenetic glucoside, which can apparently be readily extracted with hot alcohol.

The sandy clusters of crystals which separated above had the appearance and properties of pinitol. When recrystallized the melting-point (189°) and the optical activity

$$\left[\alpha\right]^{22}_{D} = \frac{+0 \cdot 57 \times 100 \times 5}{0 \cdot 5 \times 8 \cdot 75} = +65 \cdot 1^{\circ} \ (H_2O)$$

were identical with that of pinitol, the mono-methyl ester of l-inositol.

Summary.

(1) Preliminary chemical investigations upon *Lotononis laxa* E and Z, a dangerously toxic cyanogenetic plant to stock and occurring in the Lady Grey district, C.P., resulted in the isolation of a fatty ester, pinitol and benzaldehyde.

(2) The hydrocyanic-acid content of the dried and powdered plant was still very high, namely 0·26 per cent. and the view is expressed that the cyanogenetic glucoside may be constituted by the combination of a sugar, benzaldehyde and hydrocyanic-acid. Further results will follow when more of the fresh plant has been obtained.

Acknowledgement.

In conclusion I wish to thank Dr. D. G. Steyn for his interest in this research.

References.

BEILSTEIN (1920). Beilstein's Handbuch der Organischen Chemie, Vol. II, p. 373.

BERTRAND, G. (1906). *Compt. Rend.*, Vol. 143, p. 832.

FINNEMORE, H., COOPER, J. M. AND HARRIS, I. J. (1938). Mss. The Cyanogenetic Constituent of *Ximenia americana*.

FISCHER, E. AND BERGMANN (1917)., *Ber. Dtsche, chem. Ges.*, Vol 50, pp. 1047-1053.

HEILBRON, I. M. (1937). Dictionary of Organic Compounds, Vol. III, p. 329.

Section VIII.

Wool Research.

ROSSOUW, S. D. AND BOSMAN, V.	The cystine content of Merino wool in relation to its physical attributes.

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The Cystine Content of Merino Wool in Relation to its Physical Attributes.

By S. D. ROSSOUW and V. BOSMAN, Section of Wool Research, Onderstepoort.

INTRODUCTION.

THE cystine content of Merino wool is approximately 12 per cent. and although several research workers have studied the source and formation of this constituent, the function of cystine in Merino wool production from nutritional and physiological aspects has not yet been defined. In the present contribution it is not intended to deal with this aspect of the problem but to discuss the results mainly with regard to the relationships between the physical attributes of wool and its cystine content. These physical attributes largely determine the uses to which Merino wool may be put and are valuable assets either during the processes of wool manufacture or in the finished fabric. Should the cystine content of Merino wool be a modifying factor in its physical attributes, quantitative variations in cystine would have important applications in methods of wool production.

In the present investigation a series of selected Merino wool samples which differ from one another in their physical attributes has been analysed. The cystine content is compared with such characteristics as Tensile Strength, Fibre Resilience, Fibre Fineness, Crimping, Scaliness, Percentage Extension and Whiteness.

REVIEW OF LITERATURE.

Few figures are available on the correlations between the cystine content of Merino wool and its physical attributes. Several workers have analysed wool for sulphur content instead of cystine, but since it has been shown by Rimington (1929) and later by Barritt (1934) who allowed for the methionine content, that the sulphur content of wool can be converted into cystine, the relevant work dealing with the sulphur content of wool has a direct bearing on the present contribution.

Barker (1929) in discussing the ideal fabric and the manufacturing properties of wool asserted " that for an extensive series of wools of different types there is a significant variation in sulphur content, which is undoubtedly caused by biological, environmental and other influences, and which imparts to the fibre considerable variations in its response to textile processes ". After discussing the variations in the sulphur content of different wools Barker goes on to say that " it is significant that high sulphur content is accompanied by a lower regain of wool and it is certain that breed, environment and pathological condition of the animal play prominent parts in its development ". Also " a high sulphur content is desirable and it would seem to be the first difficulty in the production of our ideal fabric as to how to obtain it ".

Sidey (1931) analysed two classes of New Zealand wools for sulphur, the one lot being designated by the trade as a good processing wool and the other a fair processing lot. He found an insignificant difference between the two sets in their sulphur content.

Bonsma and Joubert (1934) working on Merino sheep found an insignificant correlation of 0·572 between the sulphur content and fibre fineness and there appeared to be no correlation between sulphur content and " quality " in Merino wool, apart from the fact that medullated fibres are inclined to be low in sulphur.

Van Wyk, Botha and Bekker (1935), while studying the effect of supplements of different forms of sulphur in the diet of Merino sheep, found that, when the animals were dosed such supplements as cystine, sulphates, KCNS and S, there was no response in their scoured fleece weights, mean fibre lengths, fibre thickness and mean fibre weights of shoulder samples. Subsequent work by Botha (unpublished) on the cystine content of the experimental wools showed no correlations between the groups, so that the abovementioned physical attributes did not appear to be correlated with the cystine content of the wools.

Smith and Harris (1936) oxidised wool artificially with hydrogen peroxide and as a result the cystine dropped from 11·6 to 8·4 per cent. They also found that " the oxidation alone showed no significant effect on the sulphur content, wet breaking strength, and resiliency of the wool ".

Swart (1936) when feeding sulphur to Merino sheep found that although the sulphur content of Merino wool was a variable quantity, no relationships could be established between sulphur content and staple length, diameter of fibre, extensibility, or crimps per inch.

McMahon and Speakman (1937) working on New Zealand Romney wool, found differences between the tips and roots of fibres in so far as their sulphur contents were concerned. They also found differences between the tips and roots of fibres in their relative degrees of " set " Their figures for variation in the sulphur content when converted into cystine content range from 11·8 to 12·6 per cent. and agree with those found in the present work.

METHODS.

Cystine was determined by the modified Sullivan method of Rossouw and Wilken-Jorden (1934). Approximately 1 gram of degreased and well-washed wool was freed of vegetable matter and sand by handpicking under distilled water with forceps in a large dish. The wool was collected on a G2 Jena fritted glass filter which was dried by suction and conditioned in the Constant Humidity chamber until constant weights were reached. The wools were then dissolved in ten times their weights of 6 N,HCl and placed in a controlled autoclave at 145° for 3 hours. It was shown that this was sufficient time for complete hydrolysis without apparent loss of cystine. The hydrolysate was filtered through G3 Jena fritted glass filters, washed and made up to suitable volume such that 5 ml. of the solution should provide approximately ·4 mg. of cystine. The colorimetric determination was carried out in the Constant Humidity Chamber at a constant temperature of 70° F. (It has been shown that a constant temperature is necessary in the colour development.) The cystine is expressed as a percentage of the clean dry wool.

In each case an average of three readings was taken for the sample, which system proved to be a reliable one. It was also shown statistically that there was a greater variation between groups constituting the average than there was within the groups, so that a real difference exists among Merino wools in their cystine contents.

Physical Attributes.

These were determined in the Constant Humidity Chamber at 70 per cent. Relative Humidity and 70° F. The methods employed and apparatus used at Onderstepoort have already been described (Bosman 1938) therefore only an outline of the methods used in this work are given here.

The tensile strength and percentage fibre extension were determined on the Doehner apparatus and the methods of sampling the wools used in the study are those described by Bosman, Waterston and van Wyk (1939).

The Fibre Resilience, expressed as the energy necessary to compress 5 grams of clean wool by 50 per cent. was determined by the Pendultex Apparatus devised by Henning. A detailed method of analysis is described by van Wyk (1939).

Fibre Fineness was determined on a Zeiss Lanameter. The crimping was measured as number of crimps per inch. Scaliness was determined by an apparatus designed after Speakman, the technique of sampling, mounting of slides and interpretation of readings being that described by Bosman and van Wyk (1939).

The degree of whiteness expressed as a percentage of standard white, was determined by the apparatus designed by Henning, where

the white light is determined by the current set up in a photo-electric cell, care being taken that all the samples were cleansed of impurities by a standardised method.

Experimental Results.

The results of the analyses are summarised in Table 1, where it is shown that the cystine content varies from 10·86 to 12·75 per cent. with a mean of 12·02 and a coefficient of variability of 3·9 per cent. This variation is comparatively small when it is compared with that of the tensile strength (7·8 per cent.) or Resilience (22·3 per cent.) or the Fibre Fineness (11·0 per cent.) or Crimps per inch (12·9 per cent.) or scaliness (16·6 per cent.) or extension at break (13·6 per cent) or Whiteness (6·8 per cent.). It must, therefore, be concluded that the cystine content of Merino wool varies less than do the physical characteristics enumerated.

Table 1.

The Cystine Content of Merino Wool in Relation to Certain of its Physical Attributes.

Sample.	Cystine Content.	Tensile Strength Gms/ CC²x10⁶	Resilience Kg/cm.	Fibre Fineness (μ).	Crimping per Inch.	Scaliness.	Extension.	Whiteness.
	Per Cent.					Per Cent.	Per Cent.	Per Cent.
47.........	10·86	0·88	1·99	17·62	10–11	76·7	78·25	57·8
25.........	12·52	1·19	5·15	23·15	13–14	45·4	61·47	54·7
37.........	11·87	1·43	3·31	22·09	10–11	67·0	69·10	59·1
21.........	11·51	1·43	2·97	21·35	10–11	79·1	69·05	56·3
48.........	11·74	0·98	2·83	19·90	13–14	76·0	78·58	56·3
62.........	12·08	1·22	3·79	26·05	10–11	73·8	83·00	59·0
111.........	11·23	1·22	2·79	18·17	12–13	65·4	51·52	58·5
120.........	11·94	1·09	3·47	22·92	11–12	61·6	58·23	52·1
29.........	12·30	1·08	4·60	25·94	10–11	52·5	80·62	65·7
14.........	12·47	1·39	5·01	17·28	—	--	52·85	55·6
51.........	11·83	0·90	3·64	19·75	15–16	55·4	89·45	53·3
45.........	11·83	1·18	3·52	--	—	—	73·83	52·9
46.........	12·60	1·12	2·10	20·26	—	64·9	83·33	58·5
20.........	11·73	1·33	3·09	21·29	9–10	86·5	69·78	54·5
22.........	11·37	1·33	3·06	21·74	9–10	82·6	67·07	53·4
32.........	11·90	1·39	4·38	18·77	12–13	65·4	70·17	64·1
71.........	12·20	1·11	3·67	24·50	12–13	65·3	65·0	54·1
5	12·75	1·13	3·16	18·36	14–15	68·5	67·75	58·0
9	12·55	1·47	3·68	20·68	12	78·4	56·92	59·1
40	12·45	1·04	2·56	20·59	9–10	81·9	68·43	48·7
35.........	11·97	1·47	3·56	21·81	9–10	—	65·15	57·2
36.........	11·90	1·53	3·40	20·72	10	61·4	65·72	57·0
27.........	12·47	1·51	4·06	24·15	12	47·7	72·83	59·1
10.........	11·95	1·48	3·88	20·56	11–12	63·6	63·68	62·4
19.........	12·45	1·47	3·85	19·62	14–15	74·6	67·75	62·4
Means.......	12·02	1·25	3·50	21·14	11·66	67·9	69·18	57·2
S.D.........	·467	·976	·781	2·409	1·499	11·242	9·428	3·906
Coeff. of V...	3·9%	7·8%	22·3%	11·0%	12·9%	16·6%	13·6%	6·8%

A comparison of the coefficients of correlation between cystine and the physical attributes are given in Table 2.

TABLE 2.

Showing Coefficient of Correlation (r).

	Tensile Strength.	Resilience.	Fibre Fineness.	Crimping.	Scaliness.	Extension.	Whiteness.
Cystine	·1714	·4509	·2181	·3281	·2242	·0705	·1026
Degrees of Freedom	23	23	22	20	20	23	23

According to Fisher's Table of r:—

	when P = ·05	when P = ·01
and when $n = 23$ then $r =$	·3951	·4999
„ $n = 20$ then r	·4227	·5368

The only significant correlation (at P = ·05) is that between cystine and resilience with a value of ·4509. The characteristics of tensile strength, Fibre Fineness, Crimping, Scaliness, Extension and Whiteness bear no definite relationships to the cystine content.

SUMMARY AND CONCLUSIONS.

A series of selected Merino wool samples that differ markedly in their physical attributes were analysed for cystine.

The results show no significant correlations between the cystine content and Tensile Strength, Fibre Fineness, Crimping, Scaliness, Percentage Extension or Whiteness. This is contrary to the findings of certain other workers.

There is a certain significant correlation (at a 5 per cent. level) between cystine content and resilience. The value of this correlation is, however, not high.

It is concluded that the rôle of cystine in wool production is not an important one, and that the possibility of improving wool characteristics via the cystine content does not appear to be great.

REFERENCES.

BARRITT, J. (1934). The distribution and origin of sulphur in wool. I. Methionine in wool. *Biochem. J.*, Vol. 28, pp. 1-5.

BONSMA, F. N., AND JOUBERT, P. J. (1934). The sulphur content of merino wool: Its distribution and relation to fineness and quality. *S.A.J.Sc.*, Vol. 31, pp. 347-370.

BOSMAN, V. (1938). Progress in the methods of wool research. *Farming in South Africa*, October, 1938.

BOSMAN, V., WATERSON, E. A., AND VAN WYK, C. M. (1939). Studies on the Tensile Strength of South African Merino Wool (Report in Preparation).

BOSMAN, V., AND VAN WYK, C. M. (1939). Studies on the Scaliness of South African Merino Wool (Report in Preparation).

McMAHON, P. R., AND SPEAKMAN, J. B. (1937). "The Action of Light on Wool." I. The titration curves of intact and exposed wools. *Trans. Far. Soc.*, Vol. 33, p. 844.

RIMINGTON, C. (1929). The relation between cystine yield and total sulphur in wool. *Biochem. J.*, Vol. 23, pp. 41-46.

ROSSOUW, S. D., AND WILKEN-JORDEN, T. J. (1934). Studies on the origin of the sulphur in wool. I. A study of the Sullivan technique for cystine. *Onderstepoort Journal*, Vol. 2, No. 1, pp. 361-368.

SIDEY, D. J. (1931). The sulphur content of some New Zealand wools. *J. Text. Inst.*, Vol. 22, T 370-373.

SMITH, A., AND HARRIS, M. (1936) The oxidation of wool. The effect of hydrogen peroxide. *Am. Dyes, Rept.*, Vol. 25, pp. 180-182.

SWART, J. C. (1936). The influence of sulphur in a ration on the growth and properties of wool. *Zeitsch. f. Zuchtung*, Reihe B, Bd. 36, h. 3, pp. 307-336.

VAN WYK, C. M., BOTHA, M. L., AND BEKKER, J. G. (1935). The effect of supplements of different forms of sulphur to the diet upon the wool of merino sheep. *Onderstepoort Journal*, Vol. 5, No. 1, pp. 177-188.

VAN WYK, C. M. (1939). Studies on the Resilience of South African Merino Wool. Report in Preparation.

VOLUME 13, No. 2.

Section I.

Protozoology.

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The Immunity in Heartwater.

By W. O. NEITZ, Section of Protozoology and Virus Diseases, Onderstepoort.

INTRODUCTION.

FIELD observations as well as immunity tests under laboratory conditions have shown that animals are capable of developing an immunity against heartwater after recovery. The nature of the immunity however is obscure. *Rickettsia ruminantium* parasitizes the endothelial cells of the blood vessels, and its presence in the peripheral blood can be demonstrated by sub-inoculation of blood into susceptible animals during the reaction and for a limited period after recovery. Alexander (1931) found that in some cases no " virus " could be demonstrated by the intra-jugular sub-inoculation of 10 c.c. blood 8 days after the height of the fever reaction, while in others transmission was successful 35 days after the reaction. In an experiment to be described later, it was possible to detect the presence of heartwater " virus " in a recovered sheep 60 days after recovery. From the data it must be concluded that a premunition may exist for a period of two months after recovery, and that the nature of the immunity after this period is obscure and needs further investigation.

In the case of *Rickettsia bovis* which parasitizes the leucocytes de Kock, van Heerden, du Toit and Neitz (1937) showed that a premunition develops, and that splenectomy results in a relapse.

A factor to be considered in these studies is the resistance of the various species of animals. As far as is known all the ruminants are susceptible to heartwater, but their susceptibility varies. The artificial infection of two species of antelopes viz. the blesbuck (*Damaliscus albifrons*) and the black wildebeest (*Conochaetes gnu*) did not result in clinical symptoms of heartwater, although the heartwater " virus " was demonstrable by sub-inoculation of blood into susceptible sheep. (Neitz 1933, 1935 and 1937.) Alexander (1931) states that indigenous sheep and blackhead-persians possess a higher degree of resistance than imported breeds. In the former breeds the mortality is approximately 6 per cent., whereas in the merino sheep up to 80 per cent. may die. Similar observations have been made with indigenous and imported breeds of goats. Insufficient information has been collected about the resistance of cattle breeds, but from field observations it would appear that one can expect the mortality to be in the neighbourhood of 60 per cent.

A third factor to be considered in these investigations is the possibility of the existence of heterologous strains. Spreull (1904), Theiler (1909) and Alexander (1931) conclude from their experiments that there are immunologically different strains. However du Toit (1924)* and the writer in these experiments have failed to confirm this statement. The observations by the former workers are summarized in Table 1 and those of the latter workers in Table 2. The immunity tests (Table 1) in the cattle, sheep and goats were applied from 3 weeks to 2 years after the recovery of the animals. In some cases the animals received virulent blood, and in other instances they were exposed to natural infection. No satisfactory explanation can be given why the earlier workers encountered immunologically different strains. A possible explanation may be that since the presence of *Eperythrozoon ovis* (Neitz, Alexander and du Toit, 1934) and that of a new " virus " which will be referred to as " virus A " in these studies was not known, incorrect conclusions may have been drawn from the febrile reactions set up by these two diseases in sheep. It will be noticed from Table 1 that no breakdown is recorded in cattle where the Eperythrozoa and the " virus A " disease do not play an important rôle, whereas in sheep and goats a large number reacted when the immunity test was applied. This argument is brought forward to show how misleading a febrile reaction may be, particularly in diseases like heartwater and " virus A " disease where the temperature may be the only symptom. In such doubtful cases therefore sub-inoculations and cross immunity tests have to be resorted to before a correct interpretation of the reaction can be given.

Observations at Onderstepoort.

For these experiments the highly susceptible merino sheep was used. The strains were passaged by sub-inoculating blood at the height of the reaction into susceptible sheep. These animals incidentally served as controls to the various experiments. No difficulty was experienced in maintaining the infection in this way, although occasionally one out of two injected sheep failed to react, in spite of the fact that such an animal was subsequently found to be fully susceptible on receiving a second infective dose. Similar observations have been recorded previously by Alexander (1931) in sheep, and by Neitz (1937) in the sub-inoculations made from infected blesbuck into sheep. The inability to transmit heartwater at times may be due to a very low concentration of the heartwater " virus " in the blood. In any of the sheep used in these experiments in which no reaction, or a doubtful one was observed, a second infective dose was given, in order to definitely determine whether the animal was susceptible or not. The mortality from the various strains was as high as 80 per cent., and consequently a relatively small number of sheep were available for these studies. Blood smears which were examined from time to time from the passage sheep during the heartwater reaction frequently showed the presence of *Eperythrozoon ovis*. These recovered animals developed a premunition to *Ep. ovis* infection, and did not react again to this disease when the

* Quoted by Alexander (1931).

TABLE 1.

Summary of the Immunity Tests by the Earlier Workers.

Animal that recovered from Heartwater.		Interval after Recovery.	Immunity Test.				
Number.	Heartwater Strain.		Heartwater Strain.	Reaction.	Result.	Author.	Year.
5 Cattle.	Not stated.............	Various intervals up to 17 months	Homologous strain injected in doses of 1000–3000 c.c. i.v.	No reaction..	Immune.....	Theiler......	1909
8 Cattle.	Not stated.............	2 years.........	Homologous strain and subsequently exposed to natural infection together with controls	No. reaction. Controls contracted heartwater	Immune.....	Theiler......	1909
3 Goats.	Not stated.............	?	Probably heterologous strain...	1 No reaction, 2 reacted	Immune. 1 died	Spreull......	1904
3 Sheep.	Not stated.............	?	Probably heterologous strain...	2 No reaction, 1 Reacted...	Immune..... Died	Spreull......	1904
8 Sheep.	Sjambokskraal and later hyperimmunized with the same strain	?	Komatipoort.................	8 Reacted...	7 Recovered. 1 Died	Theiler......	1909
8 Sheep.	Not stated.............	At various intervals up to 18 months	Homologous strain injected in doses of 50–400 c.c. i.v.	No reaction..	Immune.....	Theiler......	1909
34 Sheep.	Heartwater strain......	22–205 days....	Homologous strain............	27 No reaction 7 Reacted...	Immune.... 5 Recovered 2 Died.....	Alexander....	1929
37 Sheep.	Heartwater strain......	30–173 days....	Heterologous strain...........	24 No reaction 13 Reacted..	Immune.... 8 Recovered 5 Died.....	Alexander....	1929

heartwater immunity test was applied. On the other hand it was noticed that those sheep which contracted and recovered from a natural infection of heartwater were susceptible to *Ep. ovis*. This complicating factor can be excluded by treating the donors infected with heartwater and *Ep. ovis* with the antimony-arsenic compound Std. 386 B. 24 hours before sub-inoculating blood (Neitz 1937).

In the course of these experiments a hitherto undescribed "virus A" distinct from heartwater, blue-tongue, and tick-borne fever was isolated. This determination was important, because wrong interpretations would have been given to the reactions, if the existence of this "virus A" had not been recognized. The incubation period that follows the injection of "virus A" is as a rule longer (12-21 days) but may be as short as that of heartwater. The duration and the type of the febrile reaction are very similar to those of heartwater, and its identity can in most cases only be recognized by carrying out sub-inoculations and cross-immunity tests.

The nature of "virus A" is obscure. It can not be transmitted by the ticks *Amblyomma hebraeum*, the vector of heartwater, and *Rhipicephalus appendiculatus*. It does not pass through a Berkefeld or a Seitz filter, and does not live longer than 24 hours at room temperature in citrated blood. The mortality is extremely low and up to the present approximately 2 per cent. of the infected sheep have died. Clinically the only symptoms seen are inappetence and general weakness. At post-mortem the lesions resemble very closely those of heartwater, and the only way to differentiate these two diseases is the microscopic examination of the intima smears, and of sections of the hippocampus for the presence of *Rickettsia ruminantium*. A very important difference between "virus A" disease and heartwater is the fact that the former can be transmitted to horses, in which a mild reaction is produced after an incubation period of 18-21 days.

In the tables mentioned in Appendix 1 it will be noticed that "virus A" produced febrile reactions in heartwater immune and susceptible sheep on several occasions. This complicated the interpretations of the reactions and necessitated further sub-inoculations in order to ascertain the nature of the febrile reactions.

The experiments are discussed under two headings:—

A To determine the duration of the immunity in heartwater and to ascertain whether immunologically different strains exist.

B. To determine how this immunity is maintained.

A. TO DETERMINE THE DURATION OF THE IMMUNITY IN HEARTWATER AND TO ASCERTAIN WHETHER IMMUNOLOGICALLY DIFFERENT STRAINS EXIST.

1. Experimental Sheep.

The details of the experiments on which this discussion is based are given in Appendix 1. A large number of sheep was used and the observations on the various groups of sheep are presented in

tabular form at the end of each experiment. Another table summarizing the results of all the experiments described in Appendix 1 is given at the end of this discussion. In addition the same information is given on a chart on which the observations are demonstrated graphically. It will be seen that an insufficient number of sheep were available for each group. Nevertheless the data gives one some idea about the duration of the immunity.

For these experiments merino sheep which had recovered from an artificial infection of heartwater were used. These sheep together with a large number of others which were fully susceptible to heartwater were kept in a camp comparatively free of ticks. During the period in which they were kept in the camp no reactions and no deaths from heartwater were observed. It was therefore concluded that a natural infection of heartwater did not take place. In order to confirm this observation 13 of the susceptible sheep which are mentioned in Appendix 1 Table 8 (*a*) were used as controls to these experiments. Besides these 13 controls at least 2 susceptible sheep were included in each group of recovered heartwater sheep which were being tested for their immunity. Wherever possible the endothelial cells of the jugular veins of the control sheep that died were examined for the presence of *Rikettsia ruminantium* in order to be sure that the reactions observed were due to heartwater and not possibly due to " virus A " disease. It is of interest to mention that on several occasions it was possible to demonstrate the presence of *R. ruminantium* in the endothelial cells of the jugular veins of sheep which had died early in the evening, the next morning approximately 12 hours after death. In most of the sheep the decomposition changes were far advanced at the time when the post-mortem examination was carried out. In some instances where a sheep died late in the afternoon, and where it was not possible to carry out a post-mortem examination the same day, the jugular veins were removed and placed in a refrigerator and examined the next morning for the presence of parasites. This procedure proved very satisfactory. In one instance small portions of the jugular vein which were kept in a refrigerator were examined daily for a period of 10 days after the death of the animals. Rickettsia colonies which showed slight morphological changes could be demonstrated throughout this period. Whether they were still viable or not can not be stated as no biological test was made.

2. Origin of the Heartwater Strains.

All the ten strains mentioned below were obtained from different localities in the Transvaal. An opportunity did not present itself to study the nature of the heartwater strains which are known to occur in Natal and in the Cape Province.

(1) The strains " C.853 ", " C.860 " and " C.1024 " were isolated from three naturally infected cattle which were exposed at Onderstepoort.

(2) The strain " S.4377 " was isolated from a naturally infected sheep that contracted the disease at Onderstepoort.

(3) The "Harding" strain was isolated from a naturally infected ox in the vicinity of Pretoria.

(4) The "Krugersdorp" strain was isolated at Krugersdorp from naturally infected sheep which contracted the disease while they were on their way from the Lowveld in the Eastern Transvaal. Krugersdorp lies on the Highveld of the Transvaal, and heartwater may make its appearance there in animals that have recently been introduced from areas where heartwater is known to exist. The climatic conditions of the Highveld are such that the bont-tick (*Amblyomma hebraeum*) does not thrive there.

(5) The "Mara" strain was isolated from naturally infected sheep that were exposed on the Government Experimental Station at Mara near Louis Trichardt in the Zoutpansberg district.

(6) The "Northam" strain was isolated from naturally infected sheep which were exposed on the Government Experimental Station at Northam in the Rustenburg district.

(7) The "Strydom" strain of heartwater was isolated from naturally infected cattle on a farm in the vicinity of Warmbad in the Waterberg district.

(8) The "Zoutpansberg" strain of heartwater was isolated from naturally infected cattle in the Zoutpansberg district.

3. Summary of the Immunity and Cross Immunity Tests.

The observations made in experiments 1-9 (*b*) which are mentioned in Appendix 1 have been summarized in Table 2 of the text. It will be seen that a solid immunity was present in 121 sheep for periods up to 58 months after recovery in all of them with the exception of 10 sheep in which febrile reactions due to the heartwater test injection were noticed 7 to 34 months after recovery. No clinical symptoms other than febrile reactions were noticed. Of the 8 sheep which had recovered from the "Mara" strain febrile reactions were seen after 7 months (1 sheep), after 12 months (1 sheep), after 15 months (1 sheep), after 16 months (1 sheep); after 25 months (1 sheep), after 30 months (2 sheep) and after 34 months (1 sheep), on testing their immunity against the "Mara" heartwater strain. In one sheep which had recovered from the "S.4377" strain of heartwater a mild reaction was noticed on testing the immunity against the "Mara" strain 20 months after the primary reaction. Another sheep which recovered from both "Strydom" and "Mara" strains reacted on testing the immunity after 10 months with the "Strydom" strain of heartwater. The mild reactions indicated that there was still a partial immunity present. The cause of death in one sheep which was exposed at "Mara" six months after recovering from the "S.4377" strain could not be definitely determined. The other febrile reactions in the tested sheep were due to *Ep. ovis* in 5 cases and due to "virus A" in 5 cases.

TABLE 2.

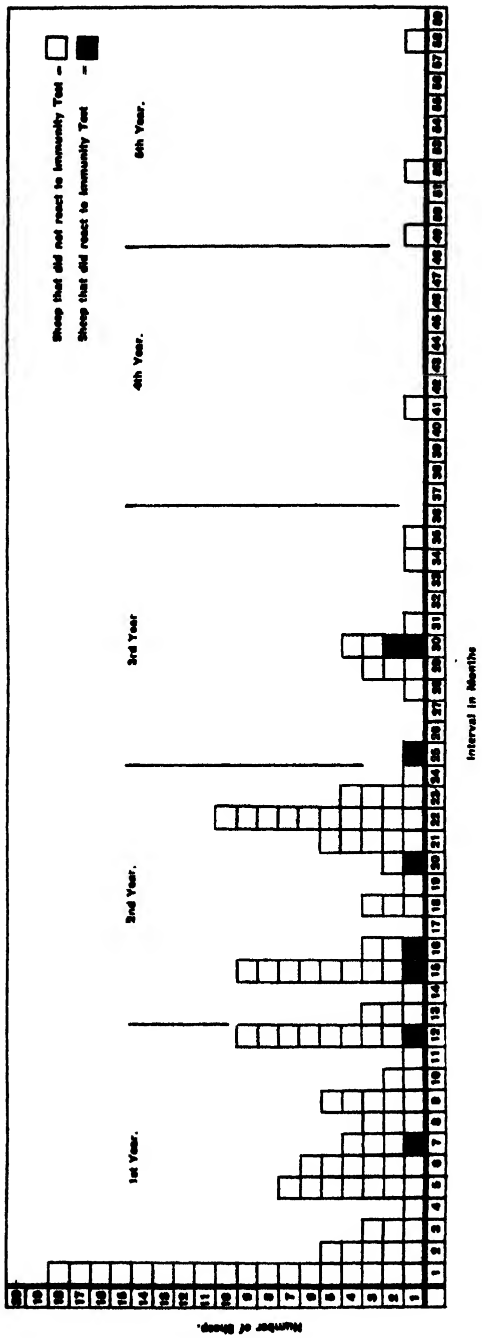
Summary of Tables 1-9.

Sheep that Recovered from Heartwater.		Approximate interval in months between the recovery and the Immunity Test.	Immunity Test.			
Number.	Strain.		Strain.	Reaction.	Result.	Table of Appendix I.
1	"Harding"	1	"Harding"	No reaction	Immune	1
3	"Harding"	2 and 12	"Zoutpansberg"	No reaction	Immune	1
1	"Harding"	5	"C. 860"	No reaction	Immune	1
1	"Harding"	5	"C. 1024"	No reaction	Immune	1
1	"Zoutpansberg"	1	"Zoutpansberg"	No reaction	Immune	1
2	"Zoutpansberg"	1	"Harding"	No reaction	Immune	1
2	"Zoutpansberg"	6	"C. 860"	No reaction	Immune	1
2	"Zoutpansberg"	5 and 6	"C. 1024"	No reaction	Immune	1
2	"Krugersdorp"	1	"C. 860"	No reaction	Immune	1
2	"Krugersdorp"	1	"C. 1024"	No reaction	Immune	1
1	"C. 853"	1	"C. 1024"	No reaction	Immune	1
1 (goat)	"C. 853"	1	"C. 1024"	No reaction	Immune	1
1	"C. 853"	2	"C. 860"	No reaction	Immune	1
1 (goat)	"C. 853"	1	"C. 860"	No reaction	Immune	1
1	"C. 853"	1	"Krugersdorp"	No reaction	Immune	1
1 (goat)	"C. 860"	1	"C. 1024"	No reaction	Immune	1
2	"C. 1024"	1 and 2	"Krugersdorp"	No reaction	Immune	1
9	"Mara"	21–23	"Mara"	No reaction	Immune	2 (a)
10	"S. 4377"	2–8	"Mara"	8 No reaction 1 Reacted 1 Abscess in lungs	Immune Died Died	2 (b) 2 (b) 2 (b)
8	"Mara" and "S. 4377"	22	"Mara"	No reaction	Immune	2 (b)
5	"Northam"	12	"Mara"	No reaction	Immune	3
2	"Mara"	2	"Northam"	No. reaction	Immune	4

TABLE 2 (*continued*).

Sheep that Recovered from Heartwater.		Approximate interval in months between the recovery and the Immunity Test.	Immunity Test.			
Number.	Strain.		Strain.	Reaction.	Result.	Table of Appendix I.
2	"Mara" and "Northam"	12	"Mara"	2 No reaction to heartwater	Immune	4
				1 Reacted to "Virus A."	Immune	4
2	"Mara"	18	"Mara"	No reaction	Immune	5
2	"Mara"	23	"Mara"	1 No reaction to heartwater	Immune	5
				1 Reacted to "Virus A."	Immune	5
3	"Mara"	12–16	"Mara"	No reaction	Immune	5
1	"Mara"	25	"Mara"	Reacted to heartwater	Recovered	5
2	"S. 4377"	20	"Mara"	1 No reaction	Immune	6
				1 Reacted to heartwater	Recovered	6
6	"S. 4377"	7–11	"Mara"	No reaction	Immune	7
6	"S. 4377" and "Mara"	15	"Mara"	No reaction	Immune	7
				1 Reacted to "Virus A."	Immune	7
				1 Reacted to heartwater	Recovered	7
5	"Mara"	3–6	"Mara"	No reaction	Immune	8
1	"Mara"	7	"Mara"	Reacted to heartwater	Recovered	8
3	"Mara"	8–9	"Mara"	No reaction	Immune	8
1	"Mara"	12	"Mara"	Reacted to heartwater	Recovered	8
7	"Mara"	13–15	"Mara"	No reaction	Immune	8
1	"Mara"	16	"Mara"	Reacted to heartwater	Recovered	8
2	"Mara"	18–19	"Mara"	No reaction	Immune	8
4	"Mara"	28–29	"Mara"	No reaction	Immune	8
4	"Mara"	30	"Mara"	2 No reaction	Immune	8
				2 Reacted to heartwater	Recovered	8
3	"Mara"	31–35	"Mara"	No reaction	Immune	8
1	"Mara"	41	"Mara"	No reaction	Immune	8
2	"Strydom" and "Mara"	9	"Strydom"	No reaction	Immune	9
1	"Strydom" and "Mara"	10	"Strydom"	Reacted to heartwater	Recovered	9
1	"Mara"	28	"Mara"	No reaction	Immune	9
1	"Mara"	34	"Mara"	Reacted to heartwater	Recovered	9
1	"Mara"	35	"Mara"	No reaction	Immune	9
1	"Mara" and "Northam"	49	"Mara"	No reaction	Immune	9
1	"Mara" and "Northam"	52	"Mara"	No reaction	Immune	9
1	"Mara"	58	"Mara"	No reaction	Immune	9

CHART INDICATING DURATION OF IMMUNITY TO HEARTWATER.



4. Discussion.

The results of the experiments indicate that once an animal has recovered from heartwater a solid immunity lasting for periods up to 58 months may be expected. In a few individual animals however only a partial immunity may be present after a period of 7 months. These observations conform with those made under field conditions. The fact that it was possible to demonstrate the presence of heartwater "virus" by sub-inoculating blood into susceptible sheep from partially immune sheep, which were reacting to the heartwater immunity test has brought forward a very important practical point in connection with the control of heartwater in the field. Should partially immune animals become re-infected with heartwater, they can act as excellent reservoirs for infecting ticks. The disease can therefore be maintained in the absence of fully susceptible animals. Mortality due to heartwater can therefore be expected on apparently heartwater free farms when susceptible stock is introduced.

In these experiments no immunological difference could be detected between the various strains employed. Reactions sometimes followed by death, have been noticed in cattle and sheep reared on heartwater veld. Such reactions should not be ascribed to a reinfection with an immunologically different strain but to a partial or a complete loss of immunity.

B. TO DETERMINE HOW THE IMMUNITY IS MAINTAINED IN HEARTWATER.

1. Summary of the Experiments Described in Appendix II.

In experiments 10 and 11 mentioned below, attempts were made to ascertain how the immunity is maintained in heartwater. In case of the bacterial diseases most of the essential aspects of immunity are known, because many methods are available for the *in vitro* and *in vivo* studies of the bacteria and their products. In case of heartwater, only *in vivo* investigations can be carried out. The cultivation of *R. ruminantium* on artificial media and the transmission of this disease to small laboratory animals have not been successful up to the present (Mason and Alexander 1938).

The blood sub-inoculations recorded by Alexander (1931) have shown that the virus may still be present 35 days after a heartwater reaction. Donatien and Lestoquard (1937) state that they were able to demonstrate the presence of heartwater "virus" in sheep 105 days after recovery. Two sheep that received large quantities of blood from a sheep 105 days after recovering from heartwater failed to react to heartwater, but a third sheep which was injected with an emulsion prepared from the endothelial cells of the blood vessels reacted to heartwater 23 days later. This animal died and at post-mortem the characteristic exudations which are seen in heartwater were observed. No attempts were made by them to confirm their diagnosis by sub-inoculation of blood into susceptible sheep or by demonstrating the presence of *R. ruminantium*.

In the experiments described in detail in Appendix II splenectomy of heartwater recovered sheep and the sub-inoculation of blood and organ emulsions were carried out in order to ascertain whether *R. ruminantium* can be demonstrated in the recovered heartwater sheep.

The results of experiment 10 can be summarized as follows:—The splenectomy of 5 recovered heartwater sheep, resulted in a relapse to either *Eperythrozoon ovis, Anaplasma ovis* or *Theileria ovis,* but not to heartwater. The rôle, if any, which is played by the spleen in maintaining the immunity in heartwater appears to be totally different to that observed in the protozoal diseases. Blood was sub-inoculated from two of the splenectomized sheep at varying intervals up to 45 days after the operation. Of the 20 sub-inoculated sheep only one reacted to heartwater, while the others reacted to *Anaplasma ovis* or *Eperythrozoon ovis.* The sheep which reacted to heartwater, had received blood from the splenectomized sheep, which had recovered from heartwater 60 days previously. The concentration of the heartwater " virus " of the splenectomized sheep must have been very low, because another sheep injected at the same time failed to react. The presence of the heartwater " virus " apparently did not stand in any relation to the splenectomy and in all probability would also have been demonstrable at that time had the spleen not been removed.

In the experiment 11 in which blood, endothelial cell scrapings of the jugular vein and emulsions prepared from the organs, were injected into 24 susceptible sheep no heartwater reactions were observed. On the contrary in three of the sub-inoculated sheep reactions due to " virus A " resulted.

2. Discussion.

Of the 44 sheep which received either blood organ emulsions or endothelial scrapings prepared from the jugular veins, only one sheep reacted to heartwater. This sheep received blood from a splenectomized sheep which had recovered from heartwater 60 days previously. In three of the heartwater recovered sheep the presence of " virus A " could be demonstrated by sub-inoculating blood into susceptible sheep. From the above results it is impossible to explain how the immunity is maintained in heartwater. If the immunity is a premunition one would have expected that several more sheep should have reacted in these tests, particularly those that were injected with endothelial cells or organ emulsions. All that is known at the present moment is that *R. ruminantium* may be present in sheep 31, 60 and 105 days after recovery. Whether this parasite is still present after this period in sheep that were found to be solidly immune after several years is not known. Another aspect of the immunity that can not be explained is that in several of the heartwater recovered sheep only a partial immunity was observed. If the immunity is due to a labile infection the question arises, why is it partial in some animals?

Before final conclusions can be drawn it is suggested that further experiments of this nature be carried out. It should however be

remembered that recovered heartwater sheep may harbour latent infections of other diseases, and that a febrile reaction that may occur in a subinoculated sheep need not necessarily be that of heartwater.

General Conclusions.

1. Studies were undertaken in order to ascertain whether immunological different strains of heartwater referred to by Spreull, Theiler and Alexander exist. For this purpose cross-immunity experiments with 10 strains obtained from different localities in the Transvaal were utilized. No difference was detected.

2. During the investigations " virus A " disease which produces a febrile reaction in sheep very similar to that of heartwater was observed on several occasions. The origin of the virus is not clear, but it would appear that a small number of the passage sheep harboured this infection. The mortality from this disease is very low viz. 2 per cent., and the lesions at post-mortem resemble very closely those of heartwater. The possibility exists that this disease may have been responsible for some of the febrile reactions and even deaths, which were noticed by the earlier workers. This suggestion is made because in the limited number of animals used in these experiments the presence of " virus A " was encountered no less than 8 times.

3. The susceptibility of the horse to " virus A " and the resistance of the horse to heartwater can be employed as a method to differentiate the two diseases, and in case of a mixed infection as a method to obtain a pure strain of " virus A ".

4. The duration of the immunity was studied in 121 sheep for a period up to 58 months after the recovery from a heartwater reaction. A solid immunity lasting for at least 6 months was observed. In the majority of cases the immunity is complete after this period, but in a very few only a partial immunity may be present at 7, 10, 12, 15, 16, 20, 25, 30 and 34 months after recovery.

5. It is suggested that partially immune animals reacting to heartwater can play an important rôle in maintaining heartwater infection in the bont-tick in the absence of fully susceptible animals.

6. Splenectomy of heartwater recoverd sheep does not result in a relapse. In one of the splenectomized sheep the presence of heartwater in the circulating blood could be demonstrated 60 days after recovering from the heartwater reaction.

7. Experiments to demonstrate the presence of *Rickettsia ruminantium* for a period longer than 60 days by the subinoculation of blood and organ emulsions from recovered heartwater sheep were entirely negative.

LITERATURE.

ALEXANDER, R. A. (1931). Heartwater: The present state of our knowledge of the disease. *17th Rept. of Dir. of Vet. Serv. and Anl. Ind. Union of S.A.*, pp. 89-150.

DE KOCK, G., VAN HEERDEN, C. J., DU TOIT, R. and NEITZ, W. O. (1937). Bovine Theileriasis in South Africa with special reference to *Theileria Mutans*. *Onderstepoort Jnl. of Vet. Sc. and Anl. Ind.*, Vol. 8, No. 1, pp. 9-125.

DONATIEN, A. and LESTOQUARD, F. (1937). État actuel des connaissances sur les Rickettsioses animales. *Arch. Inst. Pasteur d'Algérie*, Vol. 15, No. 2 pp. 142-187.

MASON, J. H. and ALEXANDER, R. A. (1938). Some aspects of rickettsial diseases of man and animals in South Africa. *Acta Conventus Tertii de Tropicis Atque Malariae Morbis.*, Part 1, pp. 526-550.

NEITZ, W. O., ALEXANDER, R. A. and DU TOIT, P. J. (1934). *Eperythrozoon ovis* a cause of anaemia in sheep. *Address Biological Society Pretoria, 15th March*, 1934.

NEITZ, W. O., ALEXANDER, R. A., DU TOIT, P. J. (1934). *Eperythrozoon ovis* (sp. nov.), Infection in sheep. *Onderstepoort Jnl. Vet. Sc. and Anl. Ind.*, Vol. 3, No. 2, pp. 263-271.

NEITZ, W. O. (1937). Eperythrozoonosis in sheep. *Onderstepoort Jnl. Vet. Sc. and Anl. Ind.*, Vol. 9, No. 1, pp. 9-30.

NEITZ, W. O. (1937). The transmission of heartwater to and from Blesbuck (*Damaliscus Albifrons*) by means of the Bont Tick (*Amblyomma hebraeum*). *Onderstepoort Jnl. of Vet. Sc. and Anl. Ind.*, Vol. 5, No. 1, pp. 37-46.

THEILER. A. (1909). Immunity in tropical and sub-tropical diseases. *The Veterinary Bacteriological Laboratories Transvaal Dept. of Agriculture*, pp. 33-35.

APPENDIX I.

A. To determine the Duration in the Immunity of Heartwater and to ascertain whether immunologically different strains exist.

Experiment 1 (S.1888. S.2142. S.2143. S.2149 *and* S.2150).

Object.—To test the immunity of sheep and goats with the same strain or a strain other than that from which the animals had recovered.

Method.—22 sheep and 2 goats which had recovered from "Harding", "Zoutpansberg", "Krugersdorp", "C.853", "C.860" and "C.1024" strains of heartwater were injected with blood from sheep reacting to the strains indicated in Table 1. The sheep used for maintaining the various strains acted as controls.

Result.—All the animals listed in Table 1 were found to be solidly immune for a period of 27 to 363 days after receiving the infective dose of heartwater blood. The control sheep all reacted to heartwater; most of them died.

Conclusion.—No immunological difference could be detected between the six strains employed in these experiments.

Experiment 2 (*a*) (S.5507).

Object.—To compare the resistance of sheep susceptible and of sheep immune to heartwater to a natural infection of heartwater.

Method.—23 susceptible sheep and 10 sheep which had recovered from an artificial infection of the heartwater strain "S.4377" were exposed at Mara in the Northern Transvaal, a locality which is known to be a very bad heartwater area.

Result.—It will be noticed from Table 2 (*a*) that of the 23 susceptible sheep 12 died from heartwater from the 20th to the 35th day after exposure, 2 showed clinical symptoms and recovered and in 9 no symptoms were observed.

TABLE 1.

Experiment 1 (S.1888. S.2142, S.2143, S.2149 and S.2150).—*To test the immunity of sheep and goats that recovered from heartwater against different strains of heartwater.*

D.O.B. Number Sheep.	Date of Injection.	Heartwater Strain.	Result.	Interval in days between date of Injection and Immunity Test.	Immunity Test. Heartwater Strain.	Immunity Test. Date of Injection.	Immunity Test. Dose of Blood i.v.	Immunity Test. Result.
6276........	13/10/23	"Harding".....	Recovered from heartwater....	363	"Zoutpansberg"	11/11/24	10 c.c.	No reaction.
8624........	22/ 9/24	"Harding".....	Recovered from heartwater....	50	"Zoutpansberg"	11/11/24	10 c.c.	No reaction.
9161........	8/ 9/24	"Harding".....	Recovered from heartwater....	65	"Zoutpansberg"	13/11/24	10 c.c.	No reaction.
8930........	13/10/24	"Harding".....	Recovered from heartwater....	29	"Harding".....	12/11/24	10 c.c.	No reaction.
8775........	9/12/24	"Harding".....	Recovered from heartwater....	158	"C. 1024.".....	16/ 5/25	5 c.c.	No reaction.
7795........	4/10/24	"Zoutpansberg"	Recovered from heartwater....	38	"Zoutpansberg"	11/11/24	10 c.c.	No reaction.
7710........	4/10/24	"Zoutpansberg"	Recovered from heartwater....	38	"Harding".....	12/11/24	10 c.c.	No reaction.
8864........	13/10/24	"Zoutpansberg"	Recovered from heartwater....	29	"Harding".....	12/11/24	10 c.c.	No reaction.
8713........	20/10/24	"Zoutpansberg"	Recovered from heartwater....	148	"C. 1024".....	16/ 5/25	5 c.c.	No reaction.
8864........	13/10/24	"Zoutpansberg"	Recovered from heartwater....	185	"C. 1024	16/ 5/25	5 c.c.	No reaction.
	12/11/24	"Zoutpansberg"	No reaction after 2nd injection					
7795........	4/10/24	"Zoutpansberg"	Recovered from heartwater....	190	"C. 860"......	20/ 5/25	5 c.c.	No reaction.
	11/11/24	"Zoutpansberg"	No reaction after 2nd injection					
8655........	31/10/24	"Zoutpansberg"	No reaction.................	168	"C. 860"......	20/ 5/25	5 c.c.	No reaction.
	3/12/24	"Harding".....	Recovered from heartwater....					
9042........	20/11/24	"Zoutpansberg"	Recovered from heartwater....	181	"C. 860"......	20/ 5/25	5 c.c.	No reaction.
10941.......	3/ 4/25	"Krugersdorp".	Recovered from heartwater....	43	"C. 1024".....	16/ 5/25	5 c.c.	No reaction.
	8/ 4/25							
11369.......	3/ 4/25	"Krugersdorp".	Recovered from heartwater....	43	"C. 1024".....	16/ 5/25	5 c.c.	No reaction.
	8/ 4/25							
11094.......	3/ 4/25	"Krugersdorp".	Recovered from heartwater....	47	"C. 860"......	20/ 5/25	5 c.c.	No reaction.
	8/ 4/25							
10684.......	23/ 4/25	"Krugersdorp".	Recovered from heartwater....	27	"C. 860"......	20/ 5/25	5 c.c.	No reaction.
10529.......	17/ 4/25	"C. 853"......	Recovered from heartwater....	29	"C. 1024".....	16/ 5/25	5 c.c.	No reaction.
10294 (goat).	31/ 3/25	"C. 853"......	Recovered from heartwater....	46	"C. 1024".....	16/ 5/25	5 c.c.	No reaction.
11112.......	31/ 3/25	"C. 853"......	Recovered from heartwater....	51	"C. 860"......	20/ 5/25	5 c.c.	No reaction.
9874 (goat).	17/ 4/25	"C. 853"......	Recovered from heartwater....	34	"C. 860"......	20/ 5/25	5 c.c.	No reaction.
10629.......	17/ 4/25	"C. 853"......	Recovered from heartwater....	34	"Krugersdorp".	20/ 5/25	5 c.c.	No reaction.
10303 (goat).	2/ 4/25	"C. 860"......	Recovered from heartwater....	44	"C. 1024".....	16/ 5/25	5 c.c.	No reaction.
6551.......	26/ 3/25	"C. 1024".....	Recovered from heartwater....	55	"Krugersdorp".	20/ 5/25	5 c.c.	No reaction.
10815.......	17/ 4/25	"C. 1024".....	Recovered from heartwater....	33	"Krugersdorp".	20/ 5/25	5 c.c.	No reaction.

Of the 10 heartwater recovered sheep mentioned in Table 2 (*b*), 8 showed no clinical symptoms, one died and at the post mortem examination showed lesions resembling those of heartwater, and another died showing multiple, localized abscesses in the lungs.

Conclusion.—The mortality in the susceptible sheep was 52 per cent. Although no clinical symptoms of heartwater were observed in 9 of the susceptible sheep exposed at Mara, it is assumed that these sheep reacted mildly to a natural infection of heartwater. It seems hardly possible that these sheep could have escaped from a natural infection if one considers the presence of the large number of bont ticks that occurred on the veld at the time. The duration of immunity of 8 of these sheep and of one which showed clinical symptoms of heartwater are discussed in the following experiment 2 (*b*). In 8 of the immune sheep the immunity was solid for a period varying from 70 to 257 days after recovering from an artificial infection of heartwater. The cause of death could not be determined in one of the sheep on account of the lack of facilities at the time in the field to carry out the necessary examination.

Experiment 2 (*b*)

Object.—To ascertain whether 9 of the sheep that survived from a natural infection at Mara are immune to heartwater for a period of approximately 21-23 months after exposure.

Method.—The 9 sheep mentioned in Table 2 (*a*) together with 2 susceptible sheep were injected intravenously with blood of the "Mara" strain of heartwater.

Result.—All the 9 sheep were found to be immune. Three of them however reacted to *Eperythrozoon ovis*, and one of them died from this infection 33 days after receiving the test dose. The 2 susceptible control sheep both reacted and died of heartwater.

Conclusions.—The immunity was solid against the homologous heartwater strain for a period of 21-23 months after exposure at Mara. *Ep. ovis* produced febrile reactions in three of these animals.

Experiment 2 (*c*).

Object.—To test the immunity of the 8 sheep, which had recovered from the "S.4377" strain of heartwater, and which had also been exposed at Mara, approximately 22 months later, against the "Mara" strain of heartwater.

Method.—These sheep together with 2 susceptible ones were injected intravenously with blood of the "Mara" strain of heartwater.

Result.—The 8 sheep referred to in Table 2 (*b*) did not react whereas both control sheep reacted and died from heartwater.

Conclusion.—The immunity was solid against the homologous strain of heartwater for a period of 22 months after exposure at Mara, and for a period of 25 to 31 months after recovering from an artificial infection with the heartwater strain "S.4377".

Experiment 3 (S.5688 *and* S.5690).

Object.—To test the immunity of 2 sheep which had recovered from a natural, and 3 sheep which had recovered from an artificial infection of the "Northam" strain of heartwater approximately 12 months after recovery.

Method.—The 5 sheep together with 2 susceptible ones were injected intravenously with blood of sheep reacting to the "Mara" strain of heartwater. The two sheep that recovered from the natural infection at Northam in the Rustenburg district reacted to *Eperythrozoon* ovis. The two control sheep both reacted and died.

Conclusions.—The 5 sheep were found to be solidly immune approximately 12 months after recovering from heartwater. No immunological difference could be found between the "Northam" and the "Mara" strains of heartwater.

TABLE 2 (a).

Experiments 2 (a) and 2 (b) (S.5507).—Sheep susceptible to heartwater exposed at Mara.

D.O.B. Number of Sheep.	Period of Exposure at Mara.	Result.	Interval in Days between First Day of Exposure at Mara and the Immunity Test.	Immunity Test.			
				Heartwater Strain.	Date of Injection.	Dose of Blood i.v.	Result.
29365	4/10/34–15/ 2/35	No reaction observed	690	"Mara"	5/ 9/36	10 c.c.	No reaction.
32798	4/10/34 15/ 2/35	No reaction observed	690	"Mara"	5/ 9/36	10 c.c.	No reaction.
37362	4/10/34–15/ 2/35	No reaction observed	626	"Mara"	23/ 6/36	10 c.c.	No reaction.
39347	4/10/34–15/ 2/35	No reaction observed	626	"Mara"	23/ 6/36	10 c.c.	Showed a febrile reaction due to *Ep. ovis*.
39414	4/10/34–15/ 2/35	No reaction observed	626	"Mara"	23/ 6/36	10 c.c.	No reaction.
39985	4/10/34–15/ 2/35	No reaction observed	626	"Mara"	23/ 6/36	10 c.c.	No reaction.
40027	4/10/34–15/ 2/35	No reaction observed	626	"Mara"	23/ 6/36	10 c.c.	Showed a febrile reaction due to *Ep. ovis*.
40126	4/10/34–15/ 2/35	Showed clinical symptoms of heartwater. 27/10/34 and recovered	676	"Mara"	12/ 8/36	10 c.c.	Showed a febrile reaction due to *Ep. ovis* and died 14/9/36, i.e. 33 days later from Eperythrozoonosis.
40375	4/10/34–15/ 2/35	No reaction observed	676	"Mara"	12/ 8/36	10 c.c.	No reaction.
38138	4/10/34–15/ 2/35	No reaction observed	—	—	—	—	—
39462	4/10/34–15/ 2/35	Showed clinical symptoms of heartwater, 27/10/34 and recovered	—	—	—	—	—
37073	4/10/34	Died from heartwater 21 days after exposure	—	—	—	—	—
37889	4/10/34	Died from heartwater 26 days after exposure	—	—	—	—	—
37911	4/10/34	Died from heartwater 20 days after exposure	—	—	—	—	—
38190	4/10/34	Died from heartwater 20 days after exposure	—	—	—	—	—
38316	4/10/34	Died from heartwater 28 days after exposure	—	—	—	—	—
38576	4/10/34	Died from heartwater 26 days after exposure	—	—	—	—	—
39640	4/10/34	Died from heartwater 28 days after exposure	—	—	—	—	—
40036	4/10/34	Died from heartwater 35 days after exposure	—	—	—	—	—
40130	4/10/34	Died from heartwater 35 days after exposure	—	—	—	—	—
40159	4/10/34	Died from heartwater 28 days after exposure	—	—	—	—	—
40162	4/10/34	Died from heartwater 18 days after exposure	—	—	—	—	—
40174	4/10/34	Died from heartwater 21 days after exposure	—	—	—	—	—

TABLE 2 (*b*).

Experiment 2 (c) (S.5507).—Sheep immnue to "S.4377" strain of heartwater exposed at Mara.

D.O.B. Number of Sheep.	Date of Injection.	Heartwater Strain.	Result.	Interval in Days between Injection and First Day of Exposure.	Period of Exposure at Mara.	Result.	Immunity Test.					
							Interval in Days between Artificial Infection and Immunity Test.	Interval in Days between First Day of Exposure and Immunity Test.	Date of Injection.	Dose of Blood i.v.	Heartwater Strain.	Result.
31788	15/7/34	"S. 4377"	Recovered from heartwater	81	4/10/34–15/2/35	No reaction observed	757	677	12/8/36	10 c.c.	"Mara"	No reaction.
37767	26/7/34	"S. 4377"	Recovered from heartwater	70	4/10/34–15/2/35	No reaction observed	747	677	12/8/36	10 c.c.	"Mara"	No reaction.
37828	4/6/34	"S. 4377"	Recovered from heartwater	122	4/10/34–15/2/35	No reaction observed	799	677	12/8/36	10 c.c.	"Mara"	No reaction.
38195	5/3/34	"S. 4377"	Recovered from heartwater	213	4/10/34–15/2/35	No reaction observed	890	677	12/8/36	10 c.c.	"Mara"	No reaction.
39531	13/4/34	"S. 4377"	Recovered from heartwater	174	4/10/34–15/2/35	No reaction observed	851	677	12/8/36	10 c.c.	"Mara"	No reaction.
37096	20/1/34	"S. 4377"	Recovered from heartwater	257	4/10/34–15/2/35	No reaction observed	934	677	12/8/36	10 c.c.	"Mara"	No reaction.
38013	15/6/34	"S. 4377"	Recovered from heartwater	111	4/10/34–15/2/35	No reaction observed	788	677	12/8/36	10 c.c.	"Mara"	No reaction.
38871	24/4/34	"S. 4377"	Recovered from heartwater	164	4/10/34–15/2/35	No reaction observed	841	677	12/8/36	10 c.c.	"Mara"	No reaction.
38921	4/4/34	"S. 4377"	Recovered from heartwater	183	4/10/34	Died 15/11/34 Cause of death was not determined	—	—	—	—	—	—
38932	24/3/34	"S. 4377"	Recovered from heartwater	194	4/10/34	Died 16/12/34 from multiple localized abscesses in the lung	—	—	—	—	—	—

TABLE 3.

Experiment 3 (S.5688 and S.5690).—Immunity test in sheep that recovered from "Northam" heartwater reaction.

D.O.B. Number of Sheep.	Date of Infection.	Heartwater Strain.	Reaction.	Interval between Heart-water Infection and Immunity Test.	Immunity Test.			
					Heart-water Strain.	Date of Injec-tion.	Dose of Blood i.v.	Result.
41839	11 and 14/6/35....	"Northam"	Recovered from heartwater	376 days	"Mara"	23/6/35	10 c.c.	No reaction.
43749	15/7/35...........	"Northam"	Recovered from heartwater	342 days	"Mara"	23/6/35	10 c.c.	No reaction.
41835	25/6/35...........	"Northam"	Recovered from heartwater	363 days	"Mara"	23/6/35	10 c.c.	No reaction.
42789	Exposed at Northam	"Northam"	Reacted to a natural infection 23/6/35 and recovered	± 365 days	"Mara"	23/6/35	10 c.c.	Showed a febrile reaction due to *Ep. ovis.*
43153	Exposed at Northam	"Northam"	Reacted to a natural infection 23/6/35 and recovered	± 365 days	"Mara"	23/6/35	10 c.c.	Showed a febrile reaction due to *Ep. ovis.*

Experiment 4 (*a*) (S.5691).

Object.—To test the immunity of 2 sheep which had recovered from an artificial infection of the "Mara" strain against the "Northam" strain of heartwater approximately 2 months after recovery.

Method.—The two recovered sheep and 2 susceptible ones were injected intravenously with blood of a sheep reacting to the "Northam" strain of heartwater.

Result.—The two sheep mentioned in Table 4 were found to be immune, whereas both the controls reacted and died.

Conclusion.—There is no immunological difference between the "Northam" and "Mara" strains of heartwater.

Experiment 4 (*b*) (S.5691).

Object.—To test the immunity of the two sheep mentioned in experiment 4 (*a*) and Table 4 for a second time with the "Mara" strain of heartwater approximately 12 months after the first immunity test.

Method.—Blood from a sheep reacting to heartwater was injected intravenously into the above-mentioned and into two susceptible sheep.

Result.—One sheep showed no reaction, whereas the other 41021 developed a febrile reaction on the 15th day. Subsequent subinoculation experiments showed that this reaction was caused by "virus A". The control sheep reacted and died of heartwater.

Conclusion.—The immunity was solid for a period of 14 months after the recovery from the "Mara" strain of heartwater, and for a period of 12 months after the first immunity test with "Northam". The febrile reaction observed in one of the sheep was caused by "virus A".

Experiment 5 (S.5507, S.5527, S.5623, S.5627, S.5961 and S.6096).

Object.—To ascertain the duration of immunity in sheep which had recovered from an artificial infection of the "Mara" strain of heartwater.

Method.—The eight sheep mentioned in Table 5, together with two susceptible ones, were injected intravenously with the "Mara" strain of heartwater.

Result.—It will be noticed that in six of the sheep no febrile reactions were noticed. One sheep 41016 showed a febrile reaction which may have been due to heartwater. This observation, however, was not confirmed by subinoculation into susceptible sheep. Sheep 47051 showed a febrile reaction due to "virus A" which disease was first noted in experiment 4 (*b*). It was not possible to demonstrate the presence of heartwater "virus" in sheep 39185, in which no reaction was observed.

Both the control sheep reacted to heartwater and died.

Conclusion.—The immunity against the homologous strain of heartwater was solid for a period of 12 to 23 months in seven of the tested sheep. In one sheep whose immunity was tested after 25 months a febrile reaction, probably due to heartwater, was noticed. The mild febrile reaction noticed in another of the sheep was due to "virus A". In an immune sheep circulating virus could not be demonstrated by subinoculation of blood.

TABLE 4.

Experiment 4 (a) and 4 (b) (S.5691).—Immunity test in sheep that recovered from "Mara" strain of heartwater.

D.O.B. Number of Sheep.	Date of Injection.	Heartwater Strain.	Result.	First Immunity Test.					Second Immunity Test.				
				Interval in Days between Injection and First Immunity Test.	Heartwater Strain.	Date of Injection.	Dose of Blood i.v.	Result.	Interval in Days between First and Second Immunity Test.	Heartwater Strain.	Date of Injection.	Dose of Blood i.v.	Result.
39435	15/5/35	"Mara"	Recovered from heartwater	54	"Northam"	8/7/35	10 c.c.	No reaction	350	"Mara"	23/6/36	10 c.c.	No reaction.
41021	23/4/35	"Mara"	Recovered from heartwater	67	"Northam"	29/6/35	10 c.c.	No reaction	359	"Mara"	23/6/36	10 c.c.	On the 15th day after injection sheep showed a febrile reaction which lasted for 14 days. The highest temperature recorded was 106·4° F. On subinoculating blood on the 4th day of reaction into sheep 45340 and 45750 it was found that the febrile reaction in sheep 41021 was due to "virus A.".

Experiment 6 (S.4377 and S.6095).

Object.—To ascertain whether sheep which had recovered from an artificial infection of the heartwater strain "S.4377" are immune to the "Mara" strain approximately 20 months after recovery.

Method.—Virulent "Mara" heartwater blood was injected intravenously into two recovered and two susceptible sheep.

Result.—The details of the results will be found in Table 6. One of the sheep was found to be solidly immune. In this animal circulating heartwater could not be demonstrated by the subinoculation of blood into susceptible sheep. The febrile reaction noted in the second sheep was found to be due to heartwater. This sheep, however, did not show any clinical symptoms.

Conclusion.—The immunity in one of the sheep was complete for a period of 20 months, and in the other a mild febrile reaction, due to heartwater, was observed. It would thus appear that although the sheep reacted a sufficient resistance or partial immunity was still present to prevent a severe attack.

Experiment 7 (*a*) (S.4377, S.5722 and S.6045).

Object.—To note the nature of immunity in sheep which had recovered from an artificial infection of heartwater strain "S.4377" by testing them with the "Mara" strain approximately seven to 11 months after recovery.

Method.—Virulent heartwater blood was injected into the six recovered and into two susceptible sheep.

Result.—It will be noticed from Table 7 that all the sheep were immune. Both controls reacted and died.

Conclusion.—The sheep were found to be fully immune for a period of seven to 11 months after recovery. No immunological difference could be detected between the "Mara" and the "S.4377" heartwater strains.

Experiment 7 (*b*).

Object.—To test the immunity of the six sheep mentioned in experiment 7 (*a*) and Table 7 for a second time against the "Mara" strain of heartwater approximately 15 months after the first immunity test.

Method.—Virulent heartwater blood was injected intravenously into the six sheep and also into two susceptible ones.

Result.—In five of the sheep no reactions were noted. In one of the animals a reaction due to "virus A" was noticed and in another a febrile reaction due to heartwater was seen

Conclusion.—The immunity was solid for a period of 20 to 25 months after the recovery from the "S.4377" strain and for a period of 15 months after the first immunity test with the "Mara" strain of heartwater. In one sheep a mild febrile reaction due to heartwater was noticed 24 months after recovery from the primary reaction and 15 months after the first immunity test.

Experiment 8.

Object.—To test the immunity of 32 sheep which had recovered from an artificial infection of the "Mara" strain of heartwater against the same strain three to 41 months after recovery.

Method.—Virulent "Mara" heartwater blood was inoculated intravenously in doses of 10 c.c. into the 32 heartwater recovered sheep mentioned in Table 8 and into 11 susceptible sheep mentioned in Table 8 (*a*).

Result.—All the susceptible sheep reacted to heartwater, and eight died. In the smears prepared from the intima of the jugular veins of the latter *Rickettsia ruminantium* could be demonstrated. Of the 32 recovered heartwater sheep, 27 showed no reaction and five showed febrile reactions, when tested after periods of seven, nine, 16 and 30 months. None of these sheep died

Conclusions.—The immunity in the majority of sheep was solid for periods up to 41 months after recovery. In only five of the sheep febrile reactions due to heartwater were noticed when tested at intervals from seven to 30 months after the primary reaction. The control sheep were found to be fully susceptible, indicating that the camp in which the sheep were allowed to run was free of infected heartwater ticks.

Experiment 9 (*a*).

Object.—To test the immunity of 9 sheep mentioned in Table 9 which had recovered from an artificial infection of the "Strydom" or "Mara" strain of heartwater 1 to 25 months after recovery from the "Mara" or the "Northam" strain of heartwater.

Method.—(1) The 3 "Strydom" heartwater recovered sheep each received 10 c.c. virulent "Mara" heartwater blood intravenously.

(2) The 4 "Mara" heartwater recovered sheep each received 10 c.c. virulent "Mara" heartwater blood intravenously.

(3) The 2 "Mara" heartwater recovered sheep each received 10 c.c. virulent "Northam" heartwater blood intravenously.

Result.—It will be seen from Table 9 that all the sheep were found to be solidly immune to the strains of heartwater used. Febrile reactions due to "virus A" disease were observed in two of the sheep which recovered from the "Strydom" strain of heartwater.

Conclusions.—The immunity was complete for periods varying from 1 to 25 months after recovery from heartwater. No immunological differences could be detected between the "Mara", "Strydom" and "Northam" strains of heartwater.

Experiment 9 (*b*).

Object.—To test the immunity of the 9 sheep mentioned in experiment 9 for periods varying from 9 to 58 months after the recovery from the infection

Method.—(1) The 3 sheep that recovered from the "Strydom" strain of heartwater and 2 susceptible sheep mentioned in Table 8 (*a*), each received 10 c.c. virulent "Strydom" heartwater blood intravenously.

(2) The 6 sheep that recovered from the "Mara" strain of heartwater each received 10 c.c. virulent "Mara" heartwater blood intravenously. The 10 susceptible sheep which were inoculated with virulent "Mara" heartwater blood in experiment 8 also served as controls in this experiment.

Result.—(1) It will be seen from Table 9 that 2 of the "Strydom" heartwater recovered sheep were solidly immune after an interval of 9 months. The third animal which was tested after 10 months showed a febrile reaction and recovered. Both the susceptible sheep injected with the "Strydom" strain of heartwater reacted. One of them died, and in the smears prepared from the intima of the jugular vein *Rickettsia ruminantium* could be demonstrated.

(2) Five of the "Mara" recovered sheep were found to be solidly immune when tested at intervals of 28 to 58 months after the primary infection. In the remaining sheep a mild febrile reaction due to heartwater was observed when tested after 34 months.

Conclusions.—Out of the 9 sheep 7 were found to be solidly immune for periods varying from 9 to 58 months. Febrile reactions were noticed in 2 sheep at 10 and 34 months after the primary reaction.

TABLE 5.

Experiment 5 (S.5507, S.5527, S.5623, S.5627, S.5961 and S.6096.—Immunity test in sheep that recovered from "Mara" strain of heartwater.

D.O.B. Number of Sheep.	Date of Injection.	Heart-water Strain.	Result.	Interval in Days between Artificial Infection and Immunity Test.	Immunity Test. Heart-water Strain.	Date of Injection.	Dose of Blood i.v.	Result.
40962	27/10/34 14/12/34	"Mara" "Mara"	Recovered from heartwater No reaction after second injection...............	710	"Mara"	4/12/36	10 c.c.	No reaction.
40184	20/ 2/35 15/ 6/35	"Mara" "Mara"	Recovered from heartwater No reaction after second injection...............	537	"Mara"	4/12/36	10 c.c.	No reaction.
41016	27/10/34	"Mara"	Recovered from heartwater	768	"Mara"	4/12/36	10 c.c.	On the 7th day after injection sheep showed a febrile reaction which lasted for 5 days. The highest temperature recorded was 107° F. This febrile reaction in all probability was due to heartwa er.
39185	11/ 4/35 15/ 5/35	"Mara" "Mara"	Recovered from heartwater No reaction after second injection..............	568 —	"Mara" —	4/12/36 —	10 c.c. —	No reaction. On the 12th day after injection 10 c.c. blood of this sheep was injected into 2 susceptible heartwater sheep 46420 and 43391 in order to ascertain whether any heartwater "virus" was circulating in the blood stream. No reactions however were noticed.
47051	22/12/34	"Mara"	Recovered from heartwater	702	"Mara"	4/12/36	10 c.c.	On the 9th day after injection sheep showed a mild febrile reaction which lasted for 4 days. The highest temperature recorded was 104° F. On the 4th day of the reaction 10c.c. blood was injected into sheep 46121 and 46691. Both showed a febrile reaction on the 16th day due to "virus A." On testing the immunity of these two sheep against heartwater they both reacted and died from heartwater.
41587	15/ 6/35	"Mara"	Recovered from heartwater	373	"Mara"	23/ 6/36	10 c.c.	No reaction.
41897	14/ 1/35	"Mara"	Recovered from heartwater	525	"Mara"	23/ 6/36	10 c.c.	No reaction.
41524	22/11/35	"Mara"	Recovered from heartwater	578	"Mara"	23/ 6/36	10 c.c.	No reaction.

TABLE 6.

Experiment 6 (*S.4377 and S.6095*).—*To test the immunity of sheep that recovered from "S.4377" strain against "Mara" strain.*

D.O.B. Number of Sheep.	Date of Injection.	Heart-water Strain.	Reaction.	Interval in Days between Infection and Immunity Test.	Immunity Test.			
					Heart-water Strain.	Date of Injection.	Dose of Blood i.v.	Reaction.
39295	2/4/35	"S. 4377"	Recovered from heartwater	610	"Mara"	3/12/36	10 c.c.	No reaction. On the 12th day after injection, blood of sheep 39295 was injected into two susceptible heartwater sheep in order to ascertain whether heartwater virus was circulating in the blood, although no febrile reaction was observed. Both subinoculated sheep 46108 and 47029 did not react.
39158	2/4/35	"S. 4377"	Recovered from heartwater	610	"Mara"	3/12/36	10 c.c.	On the 8th day after injection sheep showed a febrile reaction which lasted for 6 days. Highest temperature recorded 105 F. On the 5th day of the reaction blood was injected into sheep 46063 which reacted to heartwater and died, and into another susceptible heartwater sheep 46715 which did not react; subsequently on testing the immunity with known virulent heartwater virus sheep 46715 reacted and recovered. Sheep 39158 therefore showed a febrile reaction due to heartwater.

APPENDIX II.

B. To Determine How the Immunity is Maintained in Heartwater.

Experiment 10 (S.6123).

Object.—To ascertain whether sheep that have recovered from heartwater will show a relapse to this disease after splenectomy.

Method.—1. Five sheep which are mentioned in the appended Table 10 (*a*) were splenectomized by Dr. Quinlan, of this Institute. Two of these animals had been infected artificially with heartwater two months previously, two approximately three months previously and one which was exposed to a natural infection 16 months ago had been subsequently reinfected with blood three and a half months before it was operated on.

2. Blood from two sheep 45972 and 46053 was injected into susceptible sheep before the operation and subsequently at varying intervals up to 45 days after the removal of the spleen.

3. The temperatures were recorded twice daily.

4. Blood smears were examined daily for the appearance of blood parasites.

Result.—The details of the observations are mentioned in Tables 10 (*a*) to 10 (*c*). It will be noticed that sheep 37862 showed a relapse to *Ep.ovis*, sheep 35004 and 37362 relapsed to *A.ovis* and sheep 45972 and 46053 showed relapses to both *A.ovis* and *Th.ovis*. In none of these splenectomized animals, however, was a heartwater reaction noticed. Of the 10 sheep which were injected with blood of sheep 46053, seven reacted to *A.ovis*, one to *A.ovis* and *Ep.ovis*, and two showed no blood parasites. No reactions to heartwater were noticed in any of these sheep. A heartwater immunity test showed all the sheep to be fully susceptible.

Of the 10 sheep which had been injected with blood from 45972, six reacted to *A.ovis*, two reacted to *Ep.ovis* and *A.ovis*, one reacted to *Ep.ovis*, and one reacted to *A.ovis* and to heartwater. A heartwater immunity test gave reaction in all the sheep except in sheep 43089, which had reacted previously to heartwater after receiving blood from sheep 45972 on the ninth day after splenectomy.

Conclusion.—The splenectomy of heartwater recovered sheep resulted in a relapse to *A.ovis*, *Ep.ovis* and *Th.ovis*, but not to heartwater. The presence of circulating heartwater "virus" in sheep 45972 was demonstrated by the subinoculation of blood into sheep 43089 on the ninth day after splenectomy. The concentration of "virus" in this sheep must have been very low, because sheep 54832, which was injected at the same time as 43089, failed to react.

Experiment 11 (S.6400, S.6437 and S.6403).

Object.—To ascertain how long recovered heartwater sheep may remain carriers of *Rickettsia ruminantium*.

Method.—For this experiment three sheep which had recovered from an artificial infection with the "Mara" strain of heartwater were used as indicated in the subjoined Table 11.

(*a*) Sheep 48596 was destroyed approximately four and a half months after receiving the infective dose of heartwater blood. Organ emulsions in saline prepared from the brain, liver, kidney, spleen, the endothelial cells of the jugular vein and blood were injected into susceptible sheep.

(*b*) Sheep 48360 was destroyed approximately four months after being infected with heartwater "virus". Blood, organ emulsions, and the endothelial cells of the jugular veins were injected into sheep.

(*c*) Blood of sheep 48021 was injected into several sheep 63 and 69 days after being infected with heartwater.

Result.—(*a*) None of the sheep injected from 48596 reacted to heartwater. The two sheep which received the injection of blood, reacted to "virus A". These sheep were later found to be susceptible to heartwater. The sheep which received the organ emulsions were found to be susceptible to "virus A". Their susceptibility to heartwater was not tested.

(*b*) No heartwater reactions were observed in any of the sheep injected from 48360. Four of the sheep reacted and died as the result of a heartwater immunity test.

(*c*) The sheep injected from sheep 48021 failed to react to heartwater. In one of them, however, a reaction to "virus A" was observed. This sheep and another one were subjected to a heartwater test. Both of them reacted and recovered.

Conclusion.—It was not possible to demonstrate the presence of *Rickettsia ruminantium* by the subinoculation of blood, endothelial cells of the jugular vein and organ emulsions from the three recovered sheep after a period of 63 to 135 days after receiving an infective dose of heartwater. Two of the sheep were found to be carriers of the "virus A".

Table 10 (*a*).

The splenectomy of heartwater recovered sheep.

D.O.B. Number of Sheep.	Date of Injection.	Heartwater Reaction. Incubation Period in Days.	Heartwater Reaction. Reaction.	Interval in Days between Injection and Splenectomy.	Splenectomy. Date.	Splenectomy. Result.
35004	30/10/33 and 1/11/33........	8	Typical heartwater reaction from 6/11/33–17/11/33	98	6/ 2/34	Relapse to *A. Ovis*. No indication of a relapse to heartwater.
37862	30/10/33 and 1/11/33........	10	Typical heartwater reaction from 9/11/33–20/11/33	100	8/ 2/34	Relapse to *Ep. ovis*. No indication of a relapse to heartwater.
37362	Exposed to natural infection of heartwater at Mara from 3/10/34–15/2/35	—	No clinical symptoms seen	595	13/10/36	Relapse to *A. ovis*. No relapse to heartwater.
	Injected with heartwater blood 23/6/36		No reaction..............	112		
45972	11/11/36....................	7	Typical heartwater reaction 18–29/11/36	62	12/ 1/37	Relapse to *A. ovis* and *Th. ovis*. No relapse to heartwater. Blood subinoculated at varying intervals after splenectomy, see Table 8*b*.
46053	11/11/36....................	7	Typical heartwater reaction from 18–29/11/36	62	12/ 1/37	Relapse to *A. ovis* and *Th. ovis*. No relapse to heartwater. Blood subinoculated at varying intervals after splenectomy. See Table 8*c*.

Table 10 (*b*).

Subinoculation of blood from splenectomized sheep 45972 *mentioned in Table* 10 (*a*).

D.O.B. Number of Sheep.	Date of Injection.	Interval in days after Splenectomy.	Dose of Blood Intrav.	Incubation Period.	Result.	Heartwater Immunity Test.
42385	12/1/37	Subinoculation before splenectomy	10 c.c.	—	Reacted to *A. ovis*....	Reacted and recovered.
43395	12/1/37	Subinoculation before splenectomy	10 c.c.	—	Reacted to *A. ovis*....	Reacted and died.
43089	21/1/37	9	10 c.c.	13 days	Reacted to heartwater and recovered. Also reacted to *A. ovis*	The immunity of this sheep was tested twice but no reaction to heartwater was observed.
45832	21/1/37	9	10 c.c.	—	Reacted to *A. ovis*....	Reacted and recovered.
47047	30/1/37	18	10 c.c.	—	Reacted to *A. ovis*....	Reacted and died.
47066	30/1/37	18	10 c.c.	—	Reacted to *A. ovis* and *Ep. ovis*.	Reacted and recovered.
46210	8/2/37	27	10 c.c.	—	Reacted to *A. ovis*....	Reacted and died.
46238	8/2/37	27	10 c.c.	—	Reacted to *A. ovis*. Died from an intercurrent infection 7 weeks later	—
46194	26/2/37	45	10 c.c.	—	Reacted to *A. ovis* and *Ep. ovis*.	Reacted and died.
47040	26/2/37	45	10 c.c.	—	Reacted to *Ep. ovis*...	Reacted and died.

Table 10 (*c*).

Subinoculation of blood from splenectomized sheep 46053 mentioned in Table 10 (a).

D.O.B. Number of Sheep.	Date of Injection.	Interval in days after Splenectomy.	Dose of Blood Intravenously.	Incubation Period.	Result.	Heartwater Immunity Test.
42282	12/1/37	Subinoculation before splenectomy	10 c.c.	–	Reacted to *A. ovis*....	Reacted and died.
43091	12/1/37	Subinoculation before splenectomy	10 c.c.		Reacted to *A. ovis*....	Reacted and recovered.
45835	21/1/37	9	10 c.c.	—	Reacted to *A. ovis*....	Reacted and died.
47079	21/1/37	9	10 c.c.	--	Reacted to *A. ovis*....	Reacted and recovered.
46259	30/1/37	18	10 c.c.	–	Reacted to *A. ovis*....	Reacted and killed.
47055	30/1/37	18	10 c.c.	--	Reacted to *A. ovis*....	Reacted and died.
46430	8/2/37	27	10 c.c.	- -	Reacted to *A. ovis*....	Reacted and died.
46197	8/2/37	27	10 c.c.	—	No reaction..........	Reacted and died.
46310	26/2/37	45	10 c.c.	—	Reacted to *A. ovis* and *Ep. ovis.*	Reacted and died.
47008	26/2/37	45	10 c.c.	- -	No reaction..........	Reacted and died.

Table 11.

The injection of blood and organ emulsions of recovered heartwater sheep into susceptible sheep.

D.O.B. Number of Sheep.	Injected from.	Date.	Dose i.v.	Organ.	Result.	Remarks.
50319	48596	1/ 2/38	50 c.c	Blood	Sheep did not react to heartwater. A febrile reaction was noticed on the 14th day and lasted for 8 days. This febrile reaction was due to "virus A."	Subinoculation of blood from 50319 into sheep 50279 produced a febrile reaction not due to heartwater. On testing the immunity of sheep 50319 against the "virus A" no reaction was produced, but when injected with heartwater blood sheep reacted and recovered.
50342	48596	1/ 2/38	50 c.c	Blood	Sheep did not react to heartwater. A febrile reaction was noticed on the 14th day and lasted 12 days. This febrile reaction was due to "virus A."	Subinoculation of blood from 50342 into sheep 50291 produced a febrile reaction not due to heartwater. On testing the immunity of sheep 50342 against the "virus A." no reaction was produced, but when injected with heartwater blood it reacted and died.
50289	48596	2/ 2/38	—	Emulsion of endothelial scrapings from the jugular vein	Both sheep failed to react to heartwater	Both sheep reacted to "virus A." on testing their susceptibility.
50315	48596	2/ 2/38	—			
50280	48596	2/ 2/38	10 c.c. of a 5 per cent. emulsion	Brain	Sheep did not react to heartwater. Sheep 50280 died 5 weeks later from an intercurrent infection	Sheep 50331 was found to be susceptible to "virus A."
50331	48596	2/ 2/38				
50328	48596	2/ 2/38	10 c.c. of a 5 per cent. emulsion	Kidney	Sheep did not react to heartwater	On testing the immunity of these two sheep they were both found to be susceptible to "virus A."
50343	48596	2/ 2/38				
48044	48596	2/ 2/38	10 c.c. of a 5 per cent. emulsion	Liver	Sheep did not react to heartwater	On testing the immunity of these two sheep they were both found to be susceptible to "virus A."
50350	48596	2/ 2/38				
49945	48596	2/ 2/38	10 c.c. of a 5 per cent. emulsion	Spleen	Sheep did not react to heartwater	On testing the immunity of these two sheep they were both found to be susceptible to "virus A."
50123	48596	2/ 2/38				
40271	48360	4/11/37	50 c.c	Blood	Sheep did not react to heartwater	On testing the immunity of this sheep against heartwater it was found to be susceptible.
41952	48360	4/11/37				

Table 11 (*continued*).

D.O.B. Number of Sheep.	Injected from.	Date.	Dose i.v.	Organ.	Result.	Remarks.
47942	48360	4/11/37	—	Endoth. scrapings of the jugular vein	Sheep did not react to heartwater	—
50038	48360	4/11/37	—	Endoth. scrapings of the jugular vein	Sheep did not react to heartwater	On testing the immunity of this sheep against heartwater it was found to be susceptible.
47914	48360	4/11/37	10 c.c. of 5 per cent. emulsion	Brain, liver, spleen kidney	Sheep did not react to heartwater	—
49116	48360	4/11/37	10 c.c. of 5 per cent. emulsion	Brain, liver, spleen, kidney	Sheep did not react to heartwater	—
49910	4836C	4/11/37	10 c.c. of 5 per cent. emulsion	Brain, liver. spleen, kidney	Sheep did not react to heartwater	On testing the immunity of this sheep against heartwater it was found to be susceptible.
50253	48360	4/11/37	10 c.c. of 5 per cent. emulsion	Brain, liver, spleen, kidney	Sheep did not react to heartwater	On testing the immunity of this sheep against heartwater it was found to be susceptible.
50306	48021	3/ 2/38	10 c.c..........	Blood................	Sheep did not react to heartwater	On testing the immunity of this sheep against heartwater it was found to be susceptible.
50363	48021	3/ 2/38	10 c.c..........	Blood................	Sheep did not react to heartwater	—
50347	48021	9/ 2/38	10 c.c..........	Blood................	Sheep did not react to heartwater	—
50360	48021	9/ 2/38	10 c.c..........	Blood................	Sheep showed a febrile reaction due to "virus A."	On testing the immunity of this sheep against heartwater it was found to be susceptible.

Section II.

Virus Diseases.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 13, Number 2, October, 1939.

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A Rickettsiosis New to South Africa.

By K. SCHULZ, Section of Pathology, Onderstepoort.

THE object of this preliminary note is to record the occurrence of *Rickettsia ovina* in the monocytes in blood, intima, and lung smears of two sheep sent in during January, 1939, by the Government Veterinary Officer, Mr. J. G. de Wet, District Grootfontein, South West Africa.

The rickettsiae were more prevalent in the smears of one animal than in those of the other. A field containing two affected cells is reproduced below.

It may be of interest to mention briefly the observations recorded by Mr. de Wet regarding the above outbreak.

Severe losses, confined to the sheep only—cattle and goats not being affected—occurred on this farm over a period of about 12 months. The symptoms appeared very suddenly and animals, which had a normal appearance the previous evening, were found dead the next day. In fact a number of cases was noticed to ail only for about two hours prior to death. Not a single case suffering from this disease has yet recovered.

The animals became recumbent, lay on their side with outstretched legs, the head turned backwards and appeared to be unconscious. The eyes protruding from the orbit appeared glassy. A febrile condition was suspected.

On autopsy ticks were numerous on all sheep examined, but no heartwater producing ticks could be found. The changes noted on post mortem simulated those of heartwater to some extent, namely hydropericardium, hydrothorax, ascites, subepicardial and subendocardial haemorrhages, hyperaemia and oedema of the lungs, tumor splenis with prominent Malpighian bodies, soft and pulpy kidneys. To exclude this disease, the hippocampi of the above two sheep and those of other animals sent in at the same time were examined for *Rickettsia ruminatium*, although the officer stated that no heartwater ticks were found on the sheep in that vicinity. The result of the histological examination of this material was, however, negative for heartwater.

The former Government Veterinary Officer of Grootfontein, Dr. Sigwart, described similar symptoms in sheep on the same farm, but in addition mentioned that paralysis of the hindquarters and opisthotonus were seen in some of the sheep shortly before death. He applied the vest pocket test for cyanide poisoning with negative results. The presence of arsenic could not be demonstrated in the material sent to Onderstepoort for that purpose. He found numerous blue ticks and bontleg ticks on the sheep and stated that the veld conditions on this farm were dry and showed signs of overstocking. In addition a fairly marked verminosis consisting of wireworms, tapeworms and nodularworms was recorded in some sheep.

A blood smear sent in by him proved to be negative on microscopical examination and in the brain material of affected sheep the causal organism of heartwater could not be found.

From the above it would appear that the heavy mortality among the sheep cannot be attributed to a rickettsia infection alone, but possibly also to contributory factors such as verminosis, tick infestations and nutritional disturbances.

Much to our regret, as no further cases occurred by the time the diagnosis was made, it was impossible to investigate the outbreak further.

Lestoquard and Donatien have described *Rickettsia ovina* a parasite of the monocytes in the blood of sheep in Algiers and Anatolia. They were able to exclude anthrax, piroplasmosis and pernicious anaemia as a possible cause of the mortality. By inoculating bone-marrow and blood of affected sheep subcutaneously into susceptible ones, a febrile reaction was produced and *R. ovina* was demonstrated in the peripheral blood. Based on the results of their experiments they suggest that *Rhipicephalus bursa* is the vector of this rickettsia.

As *R. ovina* occurs exclusively in the monocytes, presenting a morphology analogous to that of *R. canis* and *R. bovis*, and as it is often frequent in the peripheral circulation, it can be easily differentiated from *R. ruminatium*, the causal organism of heartwater.

It may be mentioned here that my colleague, Mr. W. O. Neitz, was good enough to show me a preparation in which he had already found *R. ovina* in a blood smear of a sheep from the Brits area in the Transvaal prior to the arrival of the material from South West Africa. As this was a single case he naturally refrained from publishing it.

Obviously no research can be undertaken on this new condition until active outbreaks are discovered from which further material including live ticks can be obtained. This brief note is therefore published in the hopes that veterinarians in the field who encounter similar conditions will report them to enable further investigation.

REFERENCE.

DONATIEN, A. ET LESTOQUARD, F., Etat actual de connaissances sur les Rickettsioses animales. *Arch. de l'Institut Pasteur d'Algerie* XXV; 142, 1937.

Section III.

Bacteriology.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 13, Number 2, October, 1939.

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Serological Variants of *Salmonella typhi-murium* Isolated from South African Animals.

By M. W. HENNING and D. HAIG, Section of Bacteriology, Onderstepoort.

A NUMBER of epizootics in domestic animals caused by Loefflers *Bacterium typhi-murium*, containing both factors IV and V, have been described by Henning (1939). In the description given here we have confined ourselves mainly to the study of strains of *typhi-murium* lacking factor V. These were obtained from outbreaks of paratyphoid in pigeons, horses and cattle.

A. Pigeons.

Three epizootics have been investigated. Since Moore's (1895) description of a septicaemic disease in pigeons due to a bacillus of the hog cholera group several outbreaks of salmonella infection have been recorded in these birds. In most of the epizootics the disease was a paratyphoid infection *per se*, but in the outbreaks described by Zingle (1914) and Cash and Doan (1931) the body was invaded by a salmonella and the infection occurred under certain adverse conditions like pigeon-pox or alleged myeloid hyperplasia of the bone-marrow. In Holland, Reitsma (1925) and Sahaya and Willems (1927) recorded the acute form of the disease in young birds and the chronic form in adults. In Germany, Beck and Meyer (1927) and Beck (1929) described outbreaks of *typhi-murium* in adult as well as young birds, while Berge (1929) regarded this organism as the most important cause of disease in pigeons. In America, Beaudette (1926), Emmel (1929), Jungherr and Wilcox (1934) and Edwards (1935, 1938) all investigated a septicaemic disease in pigeons in which *typhi-murium* was incriminated as the cause. All pigeon strains studied by Edwards were shown to be lacking in factor V. In most countries paratyphoid is generally regarded as one of the most serious diseases of pigeons.

Outbreaks of food poisoning in man have been traced to foodstuffs derived from pigeons affected with paratyphoid. Clarenburg and Dornickx (1932) described an epidemic involving 20 persons in a hospital at the Hague. The source of infection was proved to be

pudding containing as an ingredient pigeon eggs. The outstanding symptoms were fever, diarrhoea, vomiting and gastro-enteritis. *Typhi-murium* was obtained from the blood, faeces and urine of the patients as well as from the pudding. The eggs were obtained from pigeons infected with paratyphoid and *typhi-murium* was actually isolated from some of the suspected eggs.

Outbreak I.

History.

In August, 1938, one of us (D.H.) autopsied three valuable Oriental Frills brought to the laboratory by the owner, Dr. L. V. Pearson, from Durban for investigation. About two hundred birds were kept by him in well-constructed lofts with concrete floors, while the floors of the adjoining lofts were covered with drift sand. The pigeons were valued at about £300 and approximately as much had been spent on the housing.

The pigeons, composed of different breeds, were bought mostly from a local dealer; some of these were imported from the East and had previously given negative slide agglutination tests with *gallinarum;* others were imported from leading fanciers in England. The first deaths occurred in January, 1938, when 16 birds died within a few days. Death was usually very rapid—some pigeons that appeared normal the day before were found dead the following morning; others frequently developed symptoms of vomiting and diarrhoea which lasted for a few days before the birds died. Poisoning was suspected so that a complete change was made in the food, water and feeding utensils. After a few more deaths the mortality ceased. In March, however, some fancy pigeons were bought and placed in the loft; within three days one died suddenly and three developed diarrhoea. Two of the latter died and one recovered.

In May a number of pigeons were imported from England and mixed with some valuable birds bought locally. Within a few days four of these birds had contracted a severe diarrhoea, from which all died. Periodical outbreaks occurred until March, 1939, when this paper was written. In all about eighty pigeons have died from the disease and twenty carriers have been detected by means of blood testing. Some of the latter were birds imported from England and in one case a reactor was detected on its arrival in South Africa. This proves that the infection was picked up in England and that the disease also exists in England.

The disease was usually characterised by its sudden onset and rapid ending. Several pigeons developed a green diarrhoea which generally lasted for a few days before death occurred. A few lingered for two weeks or longer and became extremely emaciated. In some cases arthritis and swelling of the joints developed, leading to lameness and dropped wing; occasionally there were indications of serious nervous disturbances, like twisting of the head, twitching of the neck and incoordination of movements. Only two of the sick birds recovered: the one was destroyed before its serum could be tested, while the other one proved to be reactor.

The following interesting observations have been made during Dr. Pearson's outbreak.

(1) Last year Dr. Pearson imported from England an Oriental Frill hen (Ring No. 2650) which had to be destroyed shortly after its arrival owing to a broken leg and wing. Before it was killed, however, an egg was laid. A Homer hen (Ring No. 378) that was used as foster parent hatched the egg and reared the young pigeon derived from the egg. Later this young Oriental Frill (Ring. No. SAPA 135) was tested and found to be a reactor. Just previously the hen (No. 378) had hatched and reared eight of her own squabs which remained healthy and proved to be non-reactors on two successive tests.

The hen (Ring No. 378) proved to be a carrier after rearing 135. Later it was destroyed and found to suffer from oophoritis. *Typhi-murium* was isolated from the affected ovary.

(2) An imported Baldhead Tumbler (Ring No. 204) acted as foster parent to two Oriental Frill squabs. She hatched and reared them until they were about 3 weeks old, when she died from paratyphoid. One of the Squabs also died from paratyphoid when about 6 weeks old. The other young pigeon (Ring No. 130) was tested as soon as it was old enough to be bled and proved to be negative for one test. Its father (Ring No. 1045) was found to be a non-reactor during two tests, while its mother (Ring No. 2006), although giving a negative reaction at the first test, proved to be positive subsequently.

(3) A Mag-pie cock (Ring No. 3406) and hen (Ring No. 3420) were paired in 1938. The hen died from paratyphoid fever in February, 1939. The cock was tested twice; the first test, in February, was negative but a subsequent one in March was positive.

(4) A couple of imported Saddle-back Tumblers, of which only the cock (Ring No. 734) was a reactor, first produced two squabs (Ring Nos. SAPA 136 and SAPA 137) both of which reacted. The one, a male (No. 137), was slaughtered but cultures made from the internal organs and intestinal contents failed to yield *typhi-murium*, the titre of the second one (No. 136) was apparently going down and the bird was being kept under observation. As a result of the second mating two more eggs were laid and hatched; one of the squabs died in its shell and the other (Ring No. SAPA 125) proved to be a reactor (titre 1 : 100). As the dead squab was not available for study, cultures could not be made and the cause of death remained unproven.

In some cases Dr. Pearson had locked in their nest-boxes, with their non-reacting mates, birds that subsequently proved to be reactors without infecting the former. There was no evidence, however, that the birds were shedding the bacteria.

There is no doubt that the disease has been kept going on the premises by a number of apparently healthy pigeons that remained carriers. By means of serological tests performed by us at different occasions twenty reactors were detected among birds that appeared

quite normal and healthy; from the organs of some of these, *typhi-murium* has been isolated. It is thought that the infection was introduced by a pigeon imported from the East and bought from a local dealer in Durban by Dr. Pearson. At the time of its introduction this bird was suffering from a form of diarrhoea, but as it was not available for examination at the time of the investigation neither serological nor cultural tests could be performed.

Autopsy of affected birds revealed extensive intestinal catarrh, swelling of the spleen and liver, necrotic nodules in the liver and lungs, oophoritis and chronic (purulent) arthritis. A pure culture of a Gram-negative, non-lactose fermenting bacterium was isolated from the heart-blood and spleen of diseased birds, and studied by one of us (M. W. H.) for its antigenic structure. The organism (labelled culture 548) was found to be motile and diphasic; it was readily agglutinated by " O " sera of group B of the Kauffmann-White schema, by *typhi-murium* type serum and by group sera. Mirror absorption tests showed that *typhi-murium* completely exhausted the agglutinins from 548 serum, but that culture 548, although removing all the type and group agglutinins from *typhi-murium* serum, merely reduced its " O " titre from 800 to 400. Moreover, *typhi-murium* var. *Copenhagen* completely exhausted 548 serum and culture 548 removed all the agglutinins from *typhi-murium* var. *Copenhagen* serum. The antigenic structure of culture 548 can be regarded, therefore, as identical with that of *typhi-murium* var. *Copenhagen*, which lacks " O " factor V.

In order to determine the extent of the infection the owner was advised to have his flock tested for carriers. Thick blood smears were taken from 150 birds and tested by the rapid agglutination method, using as antigen a thick suspension of culture 548. Seven of the slides gave definite fine, granular flocculation, and about ten gave an indefinite reaction. The owner was advised to send the seven reactors to Onderstepoort for further testing. Soon after their arrival the birds were bled and their sera used for the agglutination test (Table I).

Subsequently all the remaining birds were tested twice by the tube agglutination method with the result that a further number (14) of reactors were detected; some of these are also recorded in Table I.

According to the information recorded in Table I it will be noticed that the sera of all ten birds contained exclusively " O " agglutinins. There was no trace of either type or group agglutinin in the serum of any of the " O " reactors at a dilution of 1:20.

Nine of the pigeons were slaughtered and autopsied, and cultures were made from the internal organs (heart blood, liver, peritoneum, spleen, intestine and ovary or testes) of each one. Four of the birds were males and five females. One of the former (No. 496) was affected with chronic arthritis of the left hock and two suffered from enlarged testes (Nos. 296 and 2062), but no other pathological changes could be detected in any of the male birds. Cultures made from the pus of the affected joint of one of the male birds (No. 496) and from the liver, spleen, testes and heart-blood

of all of them remained sterile. All five of the females were affected with oophoritis. In addition, two were suffering from a mild chronic peritonitis, and one from both peritonitis and pericarditis. All five showed a variable amount of swelling of the spleen, but no apparent abnormality could be detected in the liver. Heart-blood, spleen and liver cultures made from the females yielded no growth, but a gram-negative, non-lactose fermenting organism resembling culture 548 was obtained from the ovaries of all five. A similar bacterium was obtained from a culture made from the peritoneal fluid of one bird, but not from the peritoneal fluid of the other four, nor from the pericardial fluid of the one affected with pericarditis. Cultures made from the intestinal contents of all nine pigeons yielded only lactose-fermenting Gram-negative bacteria. In all cases the material was seeded on MacConkey's bile-salt agar in Mason tubes as well as in tetrathionate broth.

TABLE I

Agglutination Reactions of the Sera of Carrier Pigeons.

Serum of.	548 "O" Antigen.	548 "type" Antigen.	548 "group" Antigen.	Result of Organ Cultures.
*Female Pigeon No. 458......	20	0	0	Ovary and heart blood pos.
Female Pigeon No. 463.......	100	0	0	Ovary, pos.
Female Pigeon No. 488.......	400	0	0	Ovary, pos.
Female Pigeon No. 492.......	200	0	0	Ovary, pos.
Male Pigeon No. 490.........	400	0	0	Negative.
Male Pigeon No. 490.........	100	0	0	Negative.
Female Pigeon No. 493.......	800	0	0	Ovary and peritoneal fluid, pos.
Male Pigeon No. 2062........	80	0	0	Negative.
Male Pigeon No. 137.........	40	0	0	Negative.
Female Pigeon No. 378.......	80	0	0	Ovary, pos.
Rabbit immunised with 548...	800	12800	6400	—

0 = less than 1 : 20; pos. = positive culture from the organ stated, cultures from all other organs being negative; negative = no growth of *typhi-murium* obtained from any organs examined.

* At a subsequent test the titre of Pigeon 458 had dropped to 1 : 10.

Although Pigeon 548, the sixth female, also gave a positive reaction when a number of birds were tested in October, 1938 (Table I) the titre of its serum was so low (1:20) that it was considered advisable to keep it under observation for some time rather than kill it for the purpose of examining its organs.

In April, 1939, this bird was again bled and its serum tested. A definite "O" agglutination was obtained only in the first tube i.e. at a dilution of 1:10. There was no "H" agglutination at all and no distinct "O" agglutination at 1:20. Approximately a week after the test the pigeon was noticed sick, it appeared listless, and sat huddled up in a corner of its cage with its feathers ruffled and was suffering from diarrhoea. The evacuations were dirty-grey

and soiled the cloacal feathers. The bird refused to eat and died after an illness which lasted about a week. The carcase was autopsied, but apart from swelling of the liver and enteritis, no pathological changes could be seen. Cultures made from the heart-blood, liver and ovary yielded a poor growth of *typhi-murium*, but no *typhi-murium* could be found in the media seeded with intestinal contents. From October, 1938, until the time of its death in May, 1939, Pigeon 548 was kept alone in a cage and did not come in contact with any other bird.

The sera of a number of obviously sick birds were tested before death, but all failed to give "O" agglutinations at dilutions of 1:20 and over.

The six strains of non-lactose fermenting bacteria isolated from the female birds were found to be actively motile and diphasic; all six strains proved to be identical with culture 548. It is significant that all six female carrier birds harboured in their ovaries *typhi-murium* var. *Copenhagen* which yielded cultures of actively motile bacteria, whereas the agglutinins found in their sera were exclusively of the "O" variety. Only six female carriers were examined and *typhi-murium* were obtained from all six.* Rabbits injected with killed suspensions of these strains of *typhi-murium* produced in their sera type, group as well as " "O" agglutinins.

This observation is in agreement with the findings of Pijper and his co-workers on typhoid fever (Pijper, 1930; Pijper and Pullinger, 1928; Pijper and Crocker, 1937). They consider that a diagnosis of enteric fever "hinges on the demonstration of 'O' agglutination". Seven of the chronic human carriers studied all gave a marked and exclusive "O" agglutination in a serum dilution of 1:100 with a sensitive strain (Ty 901). Two urinary carriers that gave only "O" agglutination produced "H" agglutinins after a series of subcutaneous injections of typhoid vaccine. They confine their search for human carriers entirely to the complement-fixation and "O" agglutination tests, completely ignoring "H" agglutination. Weil (1921) attributed the close relationship between these two tests to the fact that complement-fixation is primarily provoked by "O" antigens. In practice Daubenton (1931) confirmed Pijper's work; by simply prohibiting all African Natives with positive "O" agglutination tests from working as cooks on a goldmine he succeeded in considerably reducing the incidence of typhoid fever.

Outbreak II.

History.

This outbreak (Rogers) occurred in a flock of about 50 Homer pigeons kept in wooden lofts with the floors covered with wood shavings. Attached to the lofts is an aviary of wild birds, and in another loft about twenty yards away some fancy pigeons are kept.

* Since going to press, another four females were autopsied and *typhi-murium* was isolated from the ovary of each one.

The Homers have been kept in the present lofts for about three years. Apart from a few losses due to so-called "canker" (probably trichomoniasis) no deaths have occurred until October, 1938, when five young pigeons suddenly developed nervous symptoms associated with twisting of the neck and falling over backwards. The duration of the disease was usually about five days. One of the sick birds brought to the laboratory (Allerton) was autopsied and it showed the following lesions:—swelling of the spleen, catarrh of the intestine, and caseous purulent material in the spaces between the cerebrum and cerebellum; a Gram-negative, non-lactose fermenting bacterium (Culture 576) was isolated from this purulent material. This organism was found to be motile and diphasic; it also fell into group B of the Kauffmann-White schema. Agglutination and absorption tests showed that it resembled culture 548 in every respect; like culture 548 it lacked factor V, and its type and group antigens were identical with those of *typhi-murium*.

Outbreak III.

A third outbreak of *typhi-murium* infection in pigeons (Pietersen) was also studied by us. The birds were bought at a dispersal sale and were transported for several hundred miles to their new quarters where the disease broke out. A pure growth of *typhi-murium* (culture 629) was obtained in heart-blood, spleen and liver cultures made from several pigeons. Serological tests performed in the same way as for culture 548 showed that the bacterium is a strain of *typhi-murium* which contains both factors IV and V, differing, therefore, from the organisms isolated in the two previous outbreaks.

In the three outbreaks studied the following symptoms and lesions have been observed:—

Symptoms.

(*a*) Acute and peracute symptoms are usually manifested by young birds and sometimes by adults when the disease suddenly makes its appearance in a loft that was previously free from infection. In peracute cases, a bird that was apparently healthy the night before is found dead the following morning without having shown any symptoms of disease. In acute cases the pigeon lives for a day or two and typical symptoms have had time to develop. The bird is noticed to be off its food, it stands listless and huddled up with its feathers ruffled and it may suffer from severe diarrhoea, vomiting, thirst, pneumonia and progressive weakness. Sometimes there are nervous symptoms leading to convulsions, and paralysis of the muscles of locomotion and flight. When there has been continuous scouring the bird becomes extremely exhausted after a day or two with its vent feathers badly soiled. The mortality is always very high and recoveries are rare, but when an affected bird recovers it generally remains a carrier and so serves as a continuous source of infection. Sometimes, as in the case of Dr. Pearson's outbreak, considerable losses are sustained from this acute form of the disease in birds of all ages.

(*b*) Subacute and chronic symptoms are generally shown by adult birds. The affected pigeons frequently suffer from a form of diarrhoea which may last for weeks or even months. They usually lose condition in spite of good food and become progressively weaker and weaker until they are finally extremely emaciated before the end arrives. Birds affected with pneumonia may also live for some days before they die. Nervous symptoms and arthritis are fairly common. In this latter case the affected joints are swollen and painful, there is drooping of the wings and lameness or paralysis of the affected limb. When the nervous system is affected the birds may suffer from convulsions, twisting of the neck, incoordination of movements and paralysis of the muscles of locomotion and flight. All chronic cases should be regarded as dangerous as they may serve as reservoirs of infection as long as they remain alive. All the female reactors autopsied by us were found to be suffering from oophoritis and *typhi-murium* was isolated from the affected ovaries.

Lesions.—In acute cases there are generally indications of a septicaemia giving rise to swelling of the spleen and liver, acute catarrhal enteritis, and pneumonia; sometimes small greyish necrotic nodules are scattered throughout the lung and liver tissue, and even the pectoral muscles.

In chronic cases the carcase is usually extremely emaciated; there is softening and atrophy of the pectoral and leg muscles, and sometimes numerous greyish nodules are found in the pectoral muscles; there is arthritis with a purulent or caseo-purulent material distending the joint capsule; there may be necrotic nodules on and ulceration of the mucous membrane of the intestine, necrotic nodules in the liver and lungs, pericarditis and peritonitis. Oophoritis is common in females and males sometimes suffer from orchitis. Meningitis may be a lesion in some outbreaks.

Some infected birds may live for months and so act as carriers without showing any obvious signs of illness. Such birds are particularly dangerous as they may set up a virulent epizootic at any time when they are brought in contact with healthy birds.

Beaudette (1926) has found *typhi-murium* in the unabsorbed yolk-sacs of young birds and isolated this organism invariably from the ovaries of female carriers.

As shown above all the six female carriers autopsied by us suffered from chronic oophoritis, and *typhi-murium* was isolated from the ovary of each one. Clarenburg and Dornickx (1932) obtained *typhi-murium* from the eggs of carrier birds, but so far we have not yet succeeded in isolating the bacterium from eggs.

There are several factors which predispose the birds to infection with paratyphoid organisms, e.g. overcrowding, chilling, exposure to unhygienic conditions, transportation for long distances, infection with an intercurrent disease like pigeon-pox, or exposure to any factor that is liable to reduce the vitality and resistance of the birds.

B. Equines.

Henning and Clark (1938) described an outbreak of purulent arthritis in the foals of a stud in the Orange Free State. *Typhimurium* var. *Copenhagen* was found to be the cause of the disease. A pure culture of this organism was obtained from the pus of the affected joints. Agglutination and absorption tests were performed in the same way as with culture 548 and identical results were obtained.

According to information obtained from the manager of the stud where the outbreak occurred there is no evidence that pigeons have ever been kept in or near the stables; but the place abounds with Rock Doves, *Dialiphila phaeonota*.

C. Bovines.

More recently one of us (M.W.H.) studied an outbreak of calf-pneumonia in a dairy herd in Johannesburg. Several calves were reported sick from time to time and the majority of these died. The affected calves were weak and emaciated, lay most of the time and developed a dry cough; the breathing was usually fast and there was a dirty purulent discharge from the nose; diarrhoea was sometimes present. Three sick calves were killed and autopsied, and the following pathological changes were observed: The carcase was extremely emaciated; hard casein clots (and in one case, wood shavings) were found in the abomasum. In the first calf the liver was swollen and light yellowish-brown in colour, and several small light necrotic-looking areas were noticed on the cut surface; the spleen was slightly enlarged. In the other two cases the liver and spleen appeared normal. In all three calves the most outstanding changes were found in the thorax—there were hydrothorax and hydropericardium, and the lungs were extensively affected; there were adhesions between the costal and pulmonary pleurae, and large portions of the lung tissue were hard, consolidated and dirty in colour. On section numerous abscesses, varying in size, were exposed, the largest ones being up to 3 c.m. in diameter; irregular light-grey necrotic foci were scattered in the tissue between the abscesses. The contents of the abscesses were generally semi-fluid and varied in colour from dirty-white to slatey-blue; some of the abscesses appeared vacuolated. Cultures were made from the heart-blood, liver, spleen and lungs of all three calves, and organ specimens were taken for histological study.

In the first calf a pure culture of a Gram-negative non-lactose fermenting bacterium was obtained from the liver, spleen and heart-blood; this organism was labelled culture 580 and is described below. The culture from the lungs yielded a mixed growth of a Gram-negative organism (probably the same as culture 580), a Gram-negative, bipolar staining organism and a pleomorphic Gram-positive cocco-bacillus.

Cultures made from the organs of the second and third calves did not yield any organisms resembling those of culture 580 obtained from the first calf, but the growth from the lungs of the second calf

was composed predominently of Gram-positive diphtheroids and a few colonies of a Pasteurella. The cultures made from the lungs of the third calf yielded an apparently pure growth of Gram-positive diphtheroids.

The cocco-bacilli obtained from the three calves appeared to be identical. They refused to grow on ordinary meat-infusion agar, but on serum agar or blood agar they gave rise to a faint confluent growth after 24 hours' incubation. The individual colonies were very fine and barely visible in less than 48 hours. When the cultures were incubated for two or three days the colonies became larger and more distinct. Morphologically and culturally the organism resembles *corynebacterium pyogenes*, but a more detailed study of the bacterium is being undertaken and will form the subject of another paper. As the lung lesions presented by the three calves resemble those usually associated with *corynebacterium* infection (Lovell and Hughes, 1935) the cause of the pneumonia is provisionally attributed to this organism. The presence of the *Salmonella* (culture 580) in one calf is ascribed to secondary causes—this organism probably gained admission into the tissues of the calf after its resistance had been lowered by pneumonia due to the *corynebacterium*. A vaccine produced from culture 580 and used in the affected herd did not lower the incidence of calf pneumonia.

The significance of *pasteurellas* in the lungs of the two calves cannot be appraised at present. It is well known that *pasteurellas* frequently invade the pulmonary tissues of cattle and sheep suffering from pneumonia (Henning and Brown, 1936) but their presence may be due either to primary or secondary causes. As these *pasteurellas* were found to be non-pathogenic for mice and guinea-pigs they can be regarded as secondary invaders.

Like strain 548, culture 580 was tested against various "O", type and group sera of the Kauffmann-White schema and it was also found to be a strain of *typhi-murium*. Moreover, mirror absorption tests proved it to be a IV-variant of *typhi-murium* identical with culture 548 and, therefore, with *typhi-murium* var. *Copenhagen*. The owner of the dairy states that pigeons have never been kept on the premises and that the calves have never been off the premises. The only other record of the isolation of the IV-variant of *typhi-murium* from a bovine is that of Hohn and Harrmann (1937).

During his study of the paratyphoid B group Schutze (1920) found the two strains, Binns and Timson, serologically alike and called them the Binns type; he also showed that they were serologically related to *typhi-murium*. White (1926) failed to isolate a specific strain from *Binns* and concluded that it occurs permanently in the non-specific phase; he also noticed that it contained qualitatively a little less of the *aertrycke* "O" complex. As a result the following assignment was given to *Binns*:—IV, V: — : 1, 2, 3. After studying several strains of *Binns*, however, Edwards (1936) showed (I) that they all contained specific components characteristic of *typhi-murium* and (2) that they all lacked "O" factor V. He, therefore, amended the antigenic formula of *typhi-murium* var. *Binns* to read IV: i: 1, 2, 3. This is identical with the formula assigned by Kauffman (1934) to the organism

typhi-murium var. *Copenhagen* described by him and it is also identical with the formula given by Edwards (1935) to *typhi-murium* var. *Storrs*. But Landsteiner and Levine (1932) were the first to notice that certain strains of *typhi-murium* may be devoid of " O " factor V.

Recently several other workers have reported strains of IV-variants. Thus, Zahn (1935) found one out of 74 cultures of *typhi-murium* studied; Hoffman and Edwards (1937) and Höhn and Herrmann (1937) have isolated a number of strains of this variant from pigeons. Höhn and Herrmann have recorded one culture from a calf. Edwards (1938) studied 155 strains of *typhi-murium* of animal origin. All the IV-variants encountered were obtained either directly or indirectly from pigeons; one culture was isolated from rabbits which had been in contact with infected pigeons. All thirty cultures obtained from pigeons were IV-variants. Later Edwards studied several more IV-strains from pigeons, also one from a duck and one from a chicken.

Discussion of Biochemical Reactions.

In the biochemical tests the following points were observed:— The eight cultures from the Pearson outbreak were identical biochemically as well as serologically. Their antigenic structure resembled that of Kauffman's *S. typhi-murium* var. *Copenhagen*, but unlike the latter they all fermented inosite; their reaction to the Stern test was definitely positive while Kauffman's strain was only slightly positive. Moreover, the latter have a much stronger reaction with Jordan and Harmon's test than the Pearson strains. The Roger strain (culture 576 differed from the Pearson strains biochemically by failing to ferment inosite, but resembled them otherwise, both biochemically and serologically. The Pietersen strain (culture 629) which was a typical *typhi-murium*, containing both factors IV and V, gave a negative Stern reaction and a strongly positive Jordan and Harmon test, otherwise it resembled the Pearson strains biochemically.

The Calf strain (culture 580) resembled the Pearson strain serologically and also biochemically excepting for the fact that it gave a slightly positive Stern and a strongly positive Jordan and Harmon reaction. The Foal strain (culture 478) was a typical IV-variant serologically but biochemically it differed from the Pearson strains by giving a strong Jordan and Harmon reaction and by failing to ferment Arabinose.

The organism obtained from a chicken outbreak (culture 357), a typical *typhi-murium*, differed biochemically from two Stock strains of *typhi-murium* (Glasgow and Weybridge) by failing to react to the Bitter test and by not fermenting inosite. The two canary strains (cultures 581 and 626) resembled the two stock strains of *typhi-murium* both antigenically and biochemically.

These results show that the eight cultures of *typhi-murium* (IV-variants) isolated from the Pearson outbreak were all identical antigenically and biochemically, but that the four strains of organisms isolated from the four separate outbreaks (Pigeons 2,

TABLE 2.

Biochemical Tests of Various Strains and Variants of S. typhi-murium *and of one Strain of* Paratyphi-B.

Number and Nature of Culture.	Source.	Bitter Test.	Stern Test.	Jordan Test.	Glucose.	Lactose.	Dulcite.	Saccharose.	Mannite.	Maltose.	Arabinose.	Rhamnose.	Inulin.	Inosite.	Salicin.	Xylose.	Sorbite.	Laevulose.	Indol.	H_2S.
S. typhi-murium var. *Copenhagen* No. 548	Pigeon (Pearson).	+	+	+	+	—	+	—	+	+	+	+	—	+	—	+	+	+	—	+
S. typhi-murium v. *Copenhagen*—																				
No. 586	Pigeon (Pearson).	—	+	+	—	—	+	—	+	+	+	+	—	+	—	+	+	+	—	+
No. 588	Pigeon (Pearson).	+	+	+	+	—	+	—	+	+	+	+	—	+	—	+	+	+	—	+
No. 589	Pigeon (Pearson).	+	+	— —	+	—	—	—	+	+	+	+	—	—	—	+	+	+	—	+
No. 593	Pigeon (Pearson).	+	+	+	+	—	+	—	+	+	+	+	—	+	—	+	+	+	—	+
No. 603	Pigeon (Pearson).	+	+	+	+	—	+	—	+	+	+	+	—	+	—	+	+	+	—	+
No. 612	Pigeon (Pearson).	—	+	+ +	+	—	+	—	+	+	+	+	—	+	—	+	+	+	—	+
No. 643	Pigeon (Pearson).	—	+	+ +	+	—	+	—	+	+	+	+	—	+	—	+	+	+	—	+
No. 576	Pigeon (Rogers).	+	+	+ +	+	—	+	—	—	+	+	+	—	—	—	+	+	+	—	+
No. 580	Calf	+	±	+ + + —	+	—	—	—	+	+	+	+	—	+	—	+	+	+	—	+
S. typhi-murium—																				
No. 581	Canary	—	±	— + + +	+	—	+	—	+	—	+	+	—	+	—	+	—	+	—	+
No. 626	Canary	+	—	+ + + +	+	—	+	—	+	+	+	+	—	+	—	+	+	+	—	+
No. 629	Pigeon (Pieterson)	+	—	+ + + +	+	—	+	—	—	+	+	+	—	+	—	+	+	+	—	+
No. 527	Chicken	—	±	+ +	+	—	+	—	+	+	+	+	—	—	—	+	+	+	—	+
(Glasgow)	Stock	+*		+ + + +	+	—	+	—	+	+	+	+	—	+	—	+	+	+	—	+
(Waybridge)	Stock	+	—	+ +	—	—	+	—	+	—	+	+	—	+	—	+	+	+	—	+
F. *Storrs* (No. 19500)	Stock	+	—	— + + +	+	—	+	—	+	—	+	—	—	—	—	+	—	+	—	+
S. typhi-murium V. *Copenhagen* (No. 1147)	Stock	+	±	+ + — —	+	—	—	—	+	+	+	+	—	—	—	+	+	+	—	+
Paratyphi-B. (Scott)	Stock	—	—	—	—	—	+	—	+	—	+	+	—	+	—	+	+	—	—	+
S. typhi-murium V. *Copenhagen* No. 478	Foal	—	+	— — — —	+	—	—	—	+	+	—	+	—	+	—	+	—	+	—	+

+ = positive, — = negative, ± = slightly positive, +, + + and + + — — (in Jordan) degrees of positive reaction. *typhi-murium* var. *Copenhagen* and *typhi-murium* var. *Storrs* = *typhi-murium* var. *Binns*. Stern = Fuchsin broth according to Stern (1916). Bitter = Rhamnose medium of Bitter Weigmann and Habs (1926). Jordan = d—tartrate agar of Jordan and Harmon (1928).

Calves 1, Foals 1) were all different in their biochemical reactions, although they were all identical serologically. Moreover, not one of the four strains of *typhi-murium* (IV-variants) described by us gave the same biochemical reaction as either the Copenhagen or Storrs variety of the bacterium.

Summary.

Outbreaks of *typhi-murium* have been described in pigeons (3), foals (1) and calves (1). The antigenic structure of the different strains isolated was studied and it was shown that, with the exception of one strain from an epizootic in pigeons, all were lacking in factor V. The strain obtained from the *one* epizootic in pigeons contained both factors IV and V.

By means of agglutination tests several carriers were detected among the pigeons, but all the reactors contained exclusively " O " agglutinations—no trace of H. agglutination could be detected in any one of the carriers. All the female carriers suffered from oophoritis and actively motile strains of *typhi-murium* were obtained from all the affected ovaries.

It was not possible to isolate *typhi-murium* from the internal organs studied and intestinal contents of any one of the male carriers.

The diseases in the calves was associated with a cocco-bacillus infection, probably *Corynebacterium pyogenes*, which may be the primary cause of the pneumonia.

In foals the organism isolated from the pus of the affected joints is probably the real cause of the joint-ill.

References.

BEAUDETTE, F. R. (1926) *B. aertrycke* as an etiological agent of a disease affecting squabs. *Jl. A.V.M.A.*, Vol. 21, p. 644.

BECK, A. (1929). Beitrag zum Verhalten der Tauben-Paratyphusstämme. *Z. Inf. Haust.*, Vol. 35, p. 124.

BECK, A., AND MEYER, E. (1927). Enzootische Erkrankungen der Tauben durch Bakterien der Paratyphus-Enteritis-Gruppe. *Z. Inf. Haust.*, Vol 30, pp. 15-41.

BERGE, R. (1929) Enteritiserkrankung bei Tauben (*B. enteritis breslaviense*). *D.T.W.*, Vol. 37, pp. 247-249.

BITTER, L., WEIGMANN, F., AND HABS, H. (1926). Bestimmung der gebildeten Säuremenge zur Unterscheidung verwandter Bakterien. *Munch. Med. Wsch.*, Vol. 73, pp. 940-941.

CASH, J. R., AND DOAN, C. A. (1931). Spontaneous and experimental infection of pigeons with *B. aertrycke*. *Amer. Jl. Path.*, Vol. 7, pp. 373-397.

CLARENBURG, A., AND DORNICKX, C. G. J. (1932). Nährungsmittelvergiftung bei Menschen in Zusammenhang mit Taubenparatyphose. *Z. Hyg.*, Vol. 114, pp. 31-41.

EDWARDS, P. R. (1935). A serological variant of *S. aertrycke* isolated from pigeons. *Jl. Bact.*, Vol. 30, pp. 465-471.

EDWARDS, P. R. (1936). Serological variants of *S. typhi-murium* with special reference to *S. typhi-murium* var. *Binns*. *Jl. Hyg.*, Vol. 36, No. 3 pp. 348-354.

EDWARDS, P. R. (1938). Further studies on IV-variants of *Salmonella typhi-murium* with special reference to cultures from pigeons. *Jl. Bact.*, Vol. 35, pp. 123-128.

EMMEL, M. W. (1929). Arthritis in pigeons caused by *Salmonella schottmulleri*. *Jl. A.V.M.A.*, Vol. 28, pp. 367-370.

HENNING, M. W. (1939). The antigenic analysis of*Salmonellas* found in South African animals and birds. *Onderstepoort Jl.* (in press).

HENNING, M. W., AND BROWN, M. H. V. (1936). Pasteurellosis: An outbreak amongst sheep. Ibid., Vol. 7. No. 1, pp. 113-131.

HENNING, M. W. AND CLARK, R. (1938). Purulent arthritis in a foal due to *S. typhi-murium* var. *Copenhagen* (Storrs). *Jl. S.A. Vet. Med. Ass.*, Vol. 9, pp. 179-181.

HOFFMANN, H. A., AND EDWARDS, P. R. (1937). The spontaneous transmission of IV-variants of *S. aertrycke* from pigeons to rabbits. *Am. Jl. Hyg.*, Vol. 26, pp. 135-137.

HOHN, J., AND HERRMANN, W. (1937). Die Typen des Breslau-Bakteriums. *Z. f. Hyg.*, Vol. 119, pp. 369-382.

JORDON, E. O., AND HARMON, H. (1928). A new differential media for the paratyphoid group. *Jl. Inf. Dis.*, Vol. 42, pp. 238-241.

JUNGHERR, E., AND WILCOX, K. S. (1934). *Salmonella aertrycke* variant as an etiological agent of paratyphoid in pigeons. *Jl. Inf. Dis.*, Vol. 55, pp. 390-401.

KAUFFMANN, F. (1934). Ueber serologische und kulturelle Varianten der Paratyphus-B und Mausentyphus-Bacillen. *Z. f. Hyg.* Vol. 116, pp 368-384.

LANDSTEINER, K., AND LEVINE, P. (1932). On the Forssmann Antigens in *B. paratyphosus B and B. dysenteriae* Shiga. *Jl. Immun.*, Vol. 22, pp. 75-82.

LOVELL, R., AND HUGHES, D. L. (1935). Diseases of young calves. A Bacteriological examination of 100 cases. *Jl. Comp. Path.*, Vol. 48, pp. 267-279.

MOORE, V. A. (1895). On a pathogenic bacillus of the hog cholera group associated with a fatal disease in pigeons. *B.A.I.*, U.S.A. Bull., Vol. 8, pp. 71-78.

PIJPER, A. (1930). Serological diagnosis in Enterica. *Jl. Hyg.*, Vol. 29, pp. 380-393.

PIJPER, A., AND PULLINGER, B. D. (1928). The history of an outbreak of typhoid fever in Pretoria. *B.M.J.*, Apr. 7th

PIJPER, A. AND CROCKER, C. G. (1937). The agglutinins of typhoid fever. *Jl. S.A. Med. Ass.*, Vol. 11, pp. 113-117.

REITSMA, (1924). Quoted by Henning (1939).

SAHAYA, J., AND WILLEMS, H. (1927). A disease in pigeons due to a salmonella. *Jl. Comp. Path. and Ther.*, Vol. 40, pp. 305-8.

SCHUTZE, H. (1920). The paratyphoid B. group. *Lancet i, pp.* 93-97.

STERN, W. (1916). Studien zur Differenzierung der Bakterien der Coli-Typhus-Gruppe mittels geförbter, flüssiger Nahrboden. *Z. Bl. Bakt.*, Vol. 78, pp. 481-492.

WEIL, E. (1921). Komplementbindungsversuche. *Z. Immunitatsf.*, Vol. 31, pp. 50-84.

WHITE, P. BRUCE (1926). Further studies of the Salmonella group. *Spec. Rep. M.R.C. Series*, No. 103.

ZAHN, E. (1925). Ueber des Vo.kommen von IV-stammen bei Schottmüller und Breslaubakterien. *Z. f. Immunitätsf.*, Vol. 86, pp. 162-164.

ZINGLE, M. (1914). Untersuchungen über eine Taubenseuche mit Paratyphus-B. Bazillen-Befund. *Z. Inf. Haust.*, pp. 268-272.

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The use of Anthrax Vaccines Prepared from Avirulent (Uncapsulated) Variants of *Bacillus anthracis*.

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A TECHNIQUE has been described (Sterne 1937a, 1937b) for consistently obtaining avirulent, immunogenic variants from virulent anthrax strains. The results of field and laboratory tests with vaccines prepared from such variants will be described in this paper.

EXPERIMENTS.

To Find Out How Long Suspensions of Avirulent Variants Retained their Immunizing Power.

Avirulent variants $22A_2$, $33B_2$, $34A_2$, and $34F_2$ were grown on nutrient agar until fully sporulated. The spores were suspended in 50 per cent. glycerine-saline. Their concentration was approximately 300,000 per c.c., which is, roughly, that in vaccine prepared here ordinarily. Sheep were inoculated, subcutaneously, with 1·0 c.c. Table 1 gives the results of these laboratory tests.

TABLE 1.

Avirulent Variant	Isolated from Virulent Strain on	Vaccine prepared from Avirulent Variant on	Sheep Immunized with Vaccine on	Result of Test with Virulent Spores on: Immunized Sheep.	Result of Test with Virulent Spores on: Normal Controls.
$22A_2$............	5.2.36	13.7.36	31.7.36 22.9.36	6/6 6/6	0/4 0/4
$33B_2$............	27.7.36	13.8.36	14.8.36 7.8.37	5/6 5/6	0/4 0/4
$34A_2$............	7.8.36	13.8.36	14.8.36 7.8.37	5/6 6/6	0/4 0/4
$34F_2$............	10.8.36	10.6.38	14.6.38 12.6.39	6/8 6/6	0/2 0/2

5/6 = 5 sheep out of 6 lived.

The vaccine prepared from strain $22A_2$ was potent after nearly two months, and that prepared from strains $33B_2$, $34A_2$, and $34F_2$ after a year. It should also be noted that the vaccine from $34F_2$ was prepared two years after the isolation of this variant.

TABLE II.

Duration of Immunity Produced in Guinea-pigs by an Avirulent Variant.

Guinea Pigs.	Immunized with $34F_2$ on	Tested with 100 M.L.D. Vaccine Strain on	Result of Test on	
			Immunized Guinea Pigs.	Normal Controls.
3....................	29. 3.39	5. 6.39	3/3	0/4
5....................	25.11.38	13.12.38	5/5	0/12
3....................	25.11.38	19.12.38	3/3	0/6
3....................	25.11.38	5. 6.39	3/3	0/4

The results showed that guinea-pigs were solidly immune six months after vaccination.

Immunity Tests with Vaccines Prepared from Avirulent Variants $22A_2$ *and* $34F_2$.

All batches of avirulent vaccine, except the first, were prepared from strain $34F_2$. This was isolated from a virulent strain on 10.8.36.

Preparation of vaccine.—The strain was grown on buffered nutrient agar, pH 7·4, at 37° C. Sporulation was always complete after three days. The spores were washed off with saline and this suspension added to twice its weight of glycerine. The growth should not be washed off later than the third day, because it then adheres tenaciously to the medium. The stock glycerine-saline suspension was diluted 1:50 to 1:25 with 50 per cent. glycerine-saline for issue to the field. The dose for cattle, 1·0 c.c., contained 600,000 to 1,200,000 spores per c.c. After the middle of 1938, avirulent vaccine issued to the field was suspended in 0·5 per cent. saponin in 50 per cent. glycerine-saline. This improved the immunizing power of the vaccine. [Sterne, Robinson and Nicol (1939)]. No saponin was added for the laboratory tests. Table III summarizes the results of the laboratory titrations on sheep. Guinea-pigs could always be immunized solidly against 1 to 500 M.L.D. of a Pasteur II vaccine. These titrations are omitted for the sake of brevity.

Thus, under laboratory conditions, batches prepared from variant $34F_2$ consistently elicited a sound immunity in sheep.

TABLE III.

Strain.	Batch No.	No. of Doses Prepared.	Prepared on	Sheep Immunized on	Result of Test with Virulent Spores on	
					Immunized Sheep.	Normal Controls.
$22A_2$............	4	80,000	13.7.36	31.7.36	6/6	0/4
$34F_2$............	14	300,000	11.3.37	—	—	—
$34F_2$............	19	300,000	18.5.37	18.5.37	10/10	0/2
$34F_2$............	21	250,000	7.6.37	8.6 37	7/8	0/2
$34F_2$............	24	500,000	6 9.37	7.9.37	10/10	0/2
$34F_2$............	25	500,000	20 9 37	21.9.37	10/10	0/2
$34F_2$............	39	300,000	10.6.38	14.6.38	10/12	0/2
$34F_2$............	50	500,000	21 2.39	23.2.39	10/12	0/2
$34F_2$............	51	700,000	4.4.39	4.4.39	10/10	0/2
$34F_2$............	52	900,000	21 4.39	24.4 39	9/20	0/2
$34F_2$............	53	700,000	9.5.39	9.5.39	8/10	1/2*
$34F_2$............	54	700,000	22.5.39	22.5.39	7/10	0/2
TOTALS...............		5,730,000	—	—	97/108	1/24

* During the three years covered in Table III, 63 further normal sheep were used as controls for other batches of ordinary vaccine. All died. The one control that lived (Batch 53) showed no reaction at all, and also no reaction to a second inoculation of a large virulent test dose. It is possible that a sheep immunized previously was used here as a control.

FIELD TESTS WITH VACCINES PREPARED FROM AVIRULENT VARIANTS.

These were started in 1936, and the amount of vaccine issued was increased as experience was gained of its use. To date, June 1939, about 2,700,000 doses have been used.

Batch 4.—In 1936, 1,350 doses were issued for cattle. Reactions were inconsiderable, and a severe outbreak of anthrax was stopped.

Batch 14.—In May and June 1937, about 300,000 doses were used for cattle. There was an absence of severe reactions, even in highly-bred stock. Four animals were reported to have died, but it is doubtful whether the vaccine was responsible for the deaths.

Batch 19.—Only a few doses used.

Batches 21, 24, 25.—These were issued (1,250,000 doses) up to April 1938. By this time the avirulent vaccines had shown themselves safer than the ordinary vaccine and were being used, as a routine, for dairy cattle and higly-bred stock.

Batches 39, 50, 51.—Issued (1,100,000 doses) suspended in saponin, from April 1938 to date (June 1939). No complaints have been received.

It is difficult to produce statistically sound evidence of a vaccine's efficacy in the field. The avirulent vaccine has been tested on a large scale and its innocuousness established. The only complaints (4) of

deficiency in immunizing power concerned Batch 14. Complaints ceased when the concentration of spores was increased. The avirulent vaccine was used largely in those areas from which most of the complaints about the ordinary vaccine had come; that is in districts with a large proportion of dairy farmers. The complaints ceased when the new vaccine was introduced.

A questionnaire was sent to the farmers of one of these districts. Replies were received from 216 farmers who owned 49,000 cattle. The replies were classified as follows:—

1. *No or negligible reactions.*—200 farmers owning 46,100 cattle.
2. *Mild reactions.*—8 farmers owning 1,600 cattle. Transient drop in milk yield; 14 head reported lame.
3. *Severe reactions.*—8 farmers owning 1,300 cattle. More serious drop in milk yield; 12 animals were badly swollen; one animal died.

Questionnaires are of dubious value. Replies are usually obtained from those that are very pleased and those highly displeased. The deleterious results were certainly not all due to the vaccine, although possibly due to the inoculation procedure. There were no complaints of lack of immunity.

Comparative Field Test of Avirulent Spore Vaccine (without Saponin) and Ordinary Saponin Spore Vaccine.

Every year, in May and June, 1,700,000 cattle, the entire cattle population of the Transkei Territories, are inoculated against anthrax. In 1938 all the cattle (271,500) in one area of the Transkei were inoculated with avirulent vaccine. The cattle (1,288,030) of the rest of the Transkei were inoculated with ordinary saponin spore vaccine. Table IV gives the result of these and, for comparison, the previous year's inoculations.

TABLE IV.

Comparison of Avirulent Vaccine with Ordinary Saponin Spore Vaccine in Transkeian Territories.

Area Inoculated.	Period May–June.	No. of Cattle Inoculated.	Type of Vaccine Used.	Deaths from Anthrax in Year following Inoculation.	Percentage Deaths from Anthrax.
Butterworth.......	1937	273,730	Spore vaccine......	17	0·0062
	1938	271,500	Avirulent vaccine...	5	0·0018
Rest of Transkei..	1937	1,276,240	Spore vaccine......	60	0·0047
	1938	1,288,030	Saponin vaccine....	25	0·0019

Thus the avirulent vaccine was as effective as the ordinary saponin spore vaccine. A few years ago thousands of cattle died annually from anthrax in the Transkei. The reduction in mortality shown in Table IV is the continuation of a trend that commenced in 1928, when systematic immunization was started in the Transkei. A detailed discussion of this anti-anthrax campaign will be given in another publication.

Use of Avirulent Vaccine on Animals other than Bovines.

Sheep.—Has been used on a considerable scale; results entirely satisfactory.

Horses.—Used on a large scale in 1939; results entirely satisfactory.

Camels.—A few were inoculated and these showed no reactions.

Goats.—Under laboratory (stabled) conditions, very large doses were harmless. Under field conditions, far smaller doses provoked swellings. Of about 400 goats done in the field four died. Avirulent vaccines are not, therefore, being issued, as a routine, for goats. None of the goats that died showed anthrax septicaemia.

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SUMMARY AND CONCLUSIONS.

(1) During the last three years (1936-1939) several batches of anthrax vaccine were made from avirulent (uncapsulated) variants. These batches, as judged by laboratory tests on guinea-pigs and sheep, were uniformly good.

(2) The avirulent strains sporulated rapidly, completely and regularly.

(3) Vaccine prepared from avirulent variants retained its immunizing power for at least a year.

(4) Field tests were carried out on 2½ million cattle and several thousand horses and sheep. The vaccine was safer and produced slighter reactions that ordinary saponin spore vaccine. Highly-bred animals tolerated inoculation very well; horses and sheep could be safely inoculated with cattle vaccine.

(5) Preliminary field tests on goats indicated that these might be more affected by the uncapsulated strains than other domestic animals.

REFERENCES.

STERNE, M. (1937a). Variation in *Bacillus anthracis*. *Onderstepoort Jn. Veter. Sc and Anim. Ind.*, Vol. 8, No. 2, pp. 271-349.

STERNE, M. (1937b). Effects of different carbon dioxide concentrations on the growth of virulent anthrax strains. *Onderstepoort Jn. Veter. Sc. and Anim. Ind.*, Vol. 9, No. 1, pp. 49-67.

STERNE, M., ROBINSON, E. M., AND NICOL, J. (1939). The use of saponin spore vaccine for inoculation against anthrax in South Africa. *Onderstepoort Jnl. Veter. Sc. and Anim. Ind.*, Vol. 12, No. 2, pp.11.

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The Immunization of Laboratory Animals against Anthrax.

By MAX STERNE, Section of Bacteriology, Onderstepoort.

GUINEA-PIGS and rabbits can be immunized against anthrax without difficulty; mice as a rule cannot. The very interesting work of Tomcsik and Bodon (1934) and Tomcsik and Ivánovics (1938) on the passive immunization of mice supports Ivánovic's (1938) suggestion that mice have a unique and peculiar immunity mechanism against anthrax.

The following experiments were done to see whether mice could be actively immunized with an uncapsulated avirulent anthrax variant, and to compare their reactions with those of guinea-pigs. Uncapsulated variants have been found to immunize guinea-pigs, rabbits, goats, sheep, cattle, and horses; Stamatin and Stamatin (1936), Stamatin (1937), Sterne (1937a, 1937b, 1940).

EXPERIMENTS.

1. *The Active Immunization of Guinea-pigs and Mice against Anthrax.*

Strains used:—

(1) $34F_2$; a rough, uncapsulated, avirulent, immunizing variant isolated on 12.8.36 from a virulent strain grown on serum agar in carbon dioxide.

(2) Boiled $34F_2$; a dense suspension of (1) killed by boiling

(3) 9Ba; a rough, uncapsulated, avirulent, non-immunizing variant isolated on 30.3.35 from an avirulent, continuously dissociating, smooth mucoid strain.

Guinea-pigs and mice were immunized as shown in Table I. Their immunity was tested with 0·1 c.c. (±100 guinea-pig M.L.D.) of a glycerine-saline spore suspension of a Pasteur II strain.

TABLE I.

Immunization of Guinea-pigs and Mice against Anthrax.

Results of Tests on.	All animals tested with 100 M.L.D. (Guinea-pig) Pasteur II strain two weeks after immunization with.				
	One Dose $34F_2$.	One Dose $34F_2$ (Boiled).	One Dose 9 Ba.	Several Doses 9 Ba.	Nil (Controls).
Guinea-pigs.	59/64 (92 per cent.)	—	0/14 (0 per cent.)	0/12 (0 per cent.)	1/133 (0·8 per cent.)
Mice.......	88/160 (55 per cent.)	13/71 (23·9 per cent.)	42/147 (28·6 per cent.)	—	13/155 (8·4 per cent.)

59/64 = 59 survivors out of 64 tested.
92 % = percentage survivors.

Thus guinea-pigs immunized with $34F_2$ were almost completely resistant to the test dose, while those immunized, or hyperimmunized, with 9Ba were fully susceptible. The results got with mice were less clear. Mice immunized with $34F_2$ were significantly more resistant ($P < \cdot 01$, Fisher's χ^2 method) than those treated with either 9Ba or boiled $34F_2$. The difference between the effects of 9Ba and boiled $34F_2$ was not significant, while mice treated with either of these were significantly ($P < \cdot 01$) more resistant that the controls.

It might be argued that the more feeble response of the mouse to immunization was due to its greater susceptibility. This is not borne out by the fate of the controls. Three experiments to test the relative susceptibility to anthrax of mice and guinea-pigs are summarized below. In each experiment the mice and the guinea-pigs were inoculated with the same size dose of a Pasteur II strain.

TABLE II.

Relative Susceptibility to Anthrax of Mice and Guinea-pigs.

Experiment.	Animals Inoculated.	Results.	Mean Survival time (days) of animals that died.	Significance of difference in survival time (Fisher's t Test).
1	Guinea-pigs............	1/67	$2 \cdot 9 \pm 0 \cdot 14$	$P < \cdot 01$
	Mice..................	11/122	$5 \cdot 2 \pm 0 \cdot 23$	
2	Guinea-pigs............	0/10	$2 \cdot 3 \pm 0 \cdot 21$	$P < \cdot 01$
	Mice..................	0/42	$4 \cdot 5 \pm 0 \cdot 34$	
3	Guinea-pigs............	0/9	$2 \cdot 6 \pm 0 \cdot 20$	$P < \cdot 01$
	Mice..................	5/41	$5 \cdot 1 \pm 0 \cdot 47$	

1/67 = one animal survived of 67 inoculated.

Thus in every experiment the mice proved significantly less susceptible than guinea-pigs.

2. *The Rate of Development of Anthrax Immunity in Guinea-pigs.*

A number of guinea-pigs were inoculated with a large dose of the immunizing strain $34F_2$. Another lot received a similar dose of the non-immunizing strain 9Ba. At intervals thereafter the guinea-pigs were tested as shown in Tables III and IV. The vaccine was injected into a hind limb; the test dose into a fore limb.

TABLE III.

Rate of Development of Immunity in Guinea-pigs.

No. of Guinea-pigs.	Immunized with	Tested with 500 M.L.D. Pasteur II after	Results.
3	$34F_2$........	1 day......	† (3), † (3), † (3).
3	9Ba........	1 day......	† (3), † (3), † (3).
6	—	1 day......	† (3), † (3), † (3), † (3), † (3), † (3).
3	$34F_2$........	2 days.....	† (2), † (2), † (3).
3	9Ba........	2 days.....	† (2), † (2), † (3).
6	—	2 days	† (2), † (2), † (2), † (2), † (2), † (3).
3	$34F_2$........	4 days.....	† (3), † (4), L.
3	9Ba........	4 days	† (2), † (3), † (3).
6	—	4 days	† (2), † (2), † (2), † (2), † (3), † (3).
3	$34F_2$........	5 days.....	L, L, L.
3	9Ba........	5 days.....	† (2), † (3), † (3).
6	—	5 days.....	† (2), † (2), † (2), † (2), † (3), † (3).
2	$34F_2$........	8 days.....	L, L.
3	9Ba........	8 days.....	† (3), † (3), † (3).
3	—	8 days.....	† (2), † (2), † (3).
3	$34F_2$........	18 days.....	L, L, L.
2	9Ba........	18 days.....	† (2), † (3).
6	—	18 days.....	† (2), † (2), † (2), † (3), † (3), † (3).
3	$34F_2$........	24 days.....	L, L, L.
3	9Ba........	24 days.....	† (3), † (3), † (4).
3	—	24 days.....	† (2), † (2), † (3).

lived. † (2) = Died in two days.
non-immunized controls.

Thus immunity was shown by guinea-pigs inoculated with $34F_2$ from the 4th day, and was solid from the 5th. No immunity was ever elicited by 9Ba.

Another experiment (Table IV) was carried out to see whether immunity could be detected 24 hours after vaccination. The test dose was reduced to 100 M.L.D. Twice as much 9Ba was given as $34F_2$, as an additional control on possible non-specific effects.

TABLE IV.

Immunity of Guinea-pigs 24 Hours after Vaccination.

No. of guinea-pigs.	Immunized with	Tested with 100 M.L.D. Pasteur II strain after	Results.	Mean survival time in days of guinea-pigs that died.
39............	$34F_2$..........	24 hours.......	9/39	$4 \cdot 5 \pm 0 \cdot 55$
24............	9Ba...........	24 hours.......	0/24	$3 \cdot 2 \pm 0 \cdot 85$
26............	Controls....... (non-immunized).		0/26	$2 \cdot 6 \pm 0 \cdot 66$

There was a significantly greater number of survivors in the group immunized with strain $34F_2$. Moreover the mean survival time of the guinea-pigs that died in this group was significantly longer ($P < \cdot 01$, Fisher's *t* test) than that of the guinea-pigs in the other groups. Group 9Ba did not show a significantly longer mean survival time than that of the controls.

One may conclude that guinea-pigs inoculated with strain $34F_2$ showed a distinct and specific increase in resistance to anthrax, 24 hours after vaccination.

DISCUSSION.

Guinea-pigs and mice differed markedly in their reactions to active immunization against anthrax. The former rapidly developed a strong immunity when inoculated with the avirulent, immunizing strain $34F_2$. Some immunity was demonstrable as early as 24 hours after vaccination. This was almost certainly specific as guinea-pigs immunized or hyperimmunized with variant 9Ba showed no immunity whatever.

Mice inoculated with $34F_2$ showed a significant and considerable increase in resistance to anthrax. This, however, was not nearly so marked as in guinea-pigs. Mice inoculated with the non-immunizing strain 9Ba, or with killed suspensions of $34F_2$ also showed a significant increase in resistance. Although this increase was not as great as with $34F_2$, it was large enough to make an interpretation of the results difficult. It showed that much of the apparently specific increase in resistance produced by $34F_2$ could be non-specific. As the mice inoculated with $34F_2$ reacted more severely than those inoculated with the other strains, the influence of non-specific factors, such as inflammation, might have been correspondingly greater.

Clearly, mice are not nearly as easy to immunize actively as guinea-pigs. It may be that they never develop more than a low grade specific immunity. On the other hand mice appear more prone than guinea-pigs to develop considerable non-specific resistance. These findings could not be ascribed to the mouse's great susceptibility to anthrax, for repeated tests proved them less susceptible than guinea-pigs.

The mouse appeared to differ from other animals in its ability to develop active immunity to anthrax after inoculation with uncapsulated, immunizing variants.

Conclusions.

(1) Guinea-pigs inoculated with an avirulent, uncapsulated variant from a virulent anthrax strain rapidly developed immunity to anthrax. A significant, specific, increased resistance was detectable 24 hours after vaccination.

(2) Mice could not be immunized as easily as guinea-pigs; but showed more tendency than guinea-pigs to the production of an increased non-specific resistance.

(3) Repeated tests showed mice to be less susceptible than guinea-pigs to a test dose of approximately 100 guinea-pig M.L.D. of a Pasteur II strain.

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REFERENCES.

FISHER, R. A. (1936). Statistical methods for research workers. Oliver and Boyd, Edinburgh.

IVANOVICS, G. (1938). Ueber die Milzbrandimmunität. *Zeit. f. Immunitätsforsch.* Vol. 94. No. 5/6, pp. 436-458.

STAMATIN, N. (1937). L'Immunisation anticharbonneuse au moyen d'une souche de B. anthracis acapsulogène chez le mouton. *Compt. Rend. Soc. de Biol.*, Vol. 125, pp. 90-92.

STAMATIN, N., and L. STAMATIN (1936). Le pouvoir immunisant des souches acapsulogènes de *Bacillus anthracis.* *Compt. Rend. Soc. de Biol.* Vol. 122, pp. 491-492.

STERNE, M. (1937a). Variation in *Bacillus anthracis.* *Onderstepoort Jn. Vet. Sc. and Anim. Indust.*, Vol. 8, No. 2, pp. 271-350.

STERNE, M. (1937b). The effects of different carbon dioxide concentrations on the growth of virulent anthrax strains. *Onderstepoort Jn. Vet. Sc. and Anim. Ind.*, Vol. 9, No. 1, pp. 49-68.

STERNE, M. (1939). The use of anthrax vaccines prepared from avirulent (uncapsuled) variants of *Bacillus anthracis.* This Journal.

TOMCSIK, J., and G. BODON. (1934). Passive Schutzimpfung von Mäusen mit antikapsulärem Milzbrandimmunserum. *Wissenschaft. Woche zu Frankfurt.*, Vol. 3, pp. 114-116.

TOMCSIK, J., and G. IVANOVICS (1938). Die Schutzwirkung des Milzbrand-Antikapsel-Immunkörpers gegenüber der Milzbrandinfection. *Zeit f. Immunitätsforsch.*, Vol. 94, No. 1/2, pp. 28-44.

Section IV.

Mineral Metabolism and Deficiency.

Bisschop, J. H. R., Malan, A. I., Steyn, H. P. and Laurence, G. B.	On the feeding of a phosphorus supplement to mother-reared calves, prior to weaning, under open range conditions in Bechuanaland.

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On the Feeding of a Phosphorus Supplement to Mother-Reared Calves, prior to Weaning, under Open Range Conditions in Bechuanaland.

By J. H. R. BISSCHOP, Section of Zootechnics and Meat Research; A. I. MALAN, Section of Biochemistry; H. P. STEYN. Section of Surgery; and G. B. LAURENCE, Section of Statistics.

Introduction.

Theiler, Green and Du Toit (1924) first drew attention to a marked phosphorus deficiency in the pastures of such parts of the Union, as Bechuanaland and the Transvaal highveld. Du Toit and Bisschop (1929) showed how this deficiency materially affected growth, development, production and reproduction in cattle on the Veterinary Research Station, "Armoedsvlakte" in Bechuanaland.

Henrici (1938), Du Toit, *et. al.* (1930-1935) made an extensive survey of the feeding value of natural pasture grasses in the Union, and found a phosphorus deficiency to exist practically throughout the country. In order to prevent the effects of this deficiency, farmers were advised to supply their cattle with the necessary phosphorus supplement from the age of weaning (at 6-9 months) onwards. Prior to this age, calves of phosphorus-fed cows usually show satisfactory growth and development, and since it is a tedious process to dose young calves with bonemeal this lower age limit was introduced. For the time being the question whether or not phosphorus supplementation to calves prior to weaning, would be profitable, was left in abeyance.

At "Armoedsvlakte" a long term experiment has been in progress since 1925, in which indigenous females have been graded up systematically with purebred bulls of both indigenous and exogenous cattle breeds. In this experiment it was found that the halfbred calf generation, born out of bonemeal-fed dams, developed quite satisfactorily up to weaning age, without a phosphorus supplement. The higher bred exogenous calves, that is, the ¾ and ⅞ bred

grades out of bonemeal mothers, did not do so well as the halfbred calves up to weaning age, and the question whether such calves would not benefit by bonemeal feeding prior to weaning, had to be considered.

Object of the Experiment.

The object of the experiment was to ascertain, whether during the first 9 months of life, calves of bonemeal-fed mothers which run under open range conditions, will grow and develop more satisfactorily when supplied with a phosphorus supplement, than under the present system of management of the grade herds at " Armoedsvlakte ", i.e., where no phosphorus supplement is fed to calves until after weaning at the age of 9 months.

Plan of the Experiment.

The experiment was conducted during 1936, 1937 and 1938 at the Veterinary Research Station, " Armoedsvlakte ", near Vryburg, Cape Province, where the original researches of Theiler, Green and Du Toit were carried out 20 years ago.

A. *Experimental Animals.*

These consisted of ⅞ bred Redpoll calves, born on the Station out of ¾ bred, bonemeal-fed Redpoll cows, and sired by purebred Redpoll bulls. These calves were passed into the " Bonemeal " and " Control " groups alternately in sequence of their date of birth and sex. Table No. I gives details of the experimental animals in each group.

As will be seen from this table, one calf died in each group and a further calf had to be discharged from the Control group on account of its dam developing mastitis. The bonemeal and control groups, therefore, finally consisted respectively of 9 and 7 calves.

B. *Bonemeal Supplement.*

The " control " calves received no supplement prior to weaning. From the 271st day of age, to the end of the test at 480 days of age, they received 3 oz. of bonemeal per head per day.

The " bonemeal " calves were treated as follows:—

From date of birth to 30 days old, no supplement.

From 31 days old, to 60 days old, ½ oz. of bonemeal per day per head.

From 61 days old, to 120 days old, 1 oz. of bonemeal per day per head.

From 121 days old, to 180 days old, 2 oz. of bonemeal per day per head.

From 180 days old onwards, 3 oz. of bonemeal per day per head.

TABLE NO. I.

Bonemeal.						Control.					
No. of Calf.	Sex.	Date of Birth.	No. of Dam.	No. of Sire.	Remarks.	No. of Calf.	Sex.	Date of Birth.	No. of Dam.	No. of Sire.	Remarks.
6727	M.	24/11/36	4882	5399	—	6714	M.	18/11/36	3117	5371	—
6730	F.	26/11/36	3186	5371	—	6728	F.	24/11/36	3243	5371	—
6736	M.	2/12/36	4981	5399	—	6742	M.	7/12/36	5518	5399	—
6740	M.	4/12/36	4915	5399	—	6743	M.	7/12/36	4941	5399	—
6761	F.	17/12/36	4883	5399	—	6748	M.	8/12/36	3037	5371	—
6773	F.	28/12/36	3177	5371	—	6749	F.	9/12/36	3109	5371	—
6775	F.	28/12/36	4881	5399	Born dead.	6768	F.	26/12/36	3302	5371	—
6777	M.	30/12/36	3041	5371	—	6776	F.	29/12/36	4752	6285	Died 17/1/37. Suspected Arsenical poisoning.
6789	F.	23/ 1/37	3321	5371	—	6799	M.	12/2/37	3263	5371	1/4/37 Discharged on account of its dam, suffering from mastitis.
6790	M.	24/ 1/37	3381	5371	—						

The very young calves were dosed by opening their mouths and pouring the dry bonemeal into it. At first a few of the calves ejected some of the bonemeal, but they all soon became accustomed to this method, which worked quite satisfactorily. When the calves were older they were dosed according to standard method employed at the Station, i.e., with moistened bonemeal, by means of a spoon.

C. *Milk Rations.*

In order to obtain data concerning the milk-production of the cows, and hence concerning the milk intake of the experimental calves, it has been necessary to adopt an unusual procedure in all the experiments at "Armoedsvlakte": Where cows are dependent upon the natural pasture for their sustenance and receive no additional production ration, hand-rearing of calves is unpractical. If the calves are taken away, the cows dry up in about 10-12 weeks after parturition. Even where the calves are reared on their dams, the average lactation period is relatively short. Bonemeal fed cows usually do not give more than 2-3 lb. of milk per day after the 30th week of lactation.

The calves are allowed half their mother's milk. During the first week they suck the two right teats of the udder and the two left teats are milked for recording purposes. The following week the calves suck the left half of the udder and the right half is milked. Each Wednesday the whole udder is milked, in order to estimate whether the milk from one half, actually represents 50 per cent. of the production of the whole udder.

For the first 14 weeks (98 days) the cows are milked twice a day—for the next 16 weeks (up to and including the 210th day) only once a day. From the 211th day until weaning at 270 days of age, the calves run with their dams.

D. *Calf Management.*

The calves were brought to the milking sheds at milking times, from calf paddocks approximately 10 morgen ($\pm$ 21 acres) in extent, where there was good grazing, shelter and water supply.

E. *Body Weights.*

Body weights were taken every 30 days, from date of birth onwards until the conclusion of the experiment at 480 days of age.

F. *Pica Tests.*

Pica Tests were conducted at intervals of 14 days. The object was to determine the degree of osteophagia, or craving for bones, exhibited by the calves. The pica tests were conducted in the usual manner (see Du Toit and Bisschop, 1929) by first allowing the animals access to a trough containing sterilized rotten bones. Any animals picking up and chewing such bones were marked down as "rotten bone" cravers. The remainder were then passed into a pen containing a trough with sterilized "sweet" bones. The calves which picked up and chewed these were recorded as "sweet bone" cravers. Those that chewed neither rotten nor sweet bones were recorded as "non-cravers".

G. *Body Measurements.*

Body measurements were taken every 30 days from the dates of birth onwards. The well-known Deriaz system was employed. The measurements taken were:—

(*a*) Length of body.
(*b*) Height at withers.
(*c*) Height at hookbones.
(*d*) Depth of chest.
(*e*) Width of chest.
(*f*) Length of rump.
(*g*) Width between hookbones.
(*h*) Width between thirls.
(*i*) Width between pinbones.
(*j*) Length of head.
(*k*) Width between eyes.
(*l*) Heart girth or body circumference.

H. *Blood Analysis.*

A blood sample was taken from each calf at the ages of 30, 60, 120 and 180 days of age and analysed for inorganic phosphorus. Thereafter all the experimental animals were bled for similar tests once a month from October 1937 to March 1938, i.e. from the approximate average ages of 11 months to 16 months.

I. *Histological Studies of Bones.*

The experimental programme called for rib-resections at the costo-chondral junction, of all the experimental calves at the ages of 90 and 210 days, but on account of the time required for a number of these operations, it was decided to select one heifer and one "tolly calf"[1] from each group. These 4 calves were operated on in the middle of February and again in the middle of June 1937, approximately at the ages of 65 and 180 days. The resected pieces of ribs were examined histologically to ascertain whether calcification was taking place normally and at the same rate, in both groups.

J. *Statistical Analysis of Data.*

All data were analysed statistically according to Fisher (1936). Only such differences which proved to be significant in terms of Fishers $P = 0 \cdot 05$ or $P = 0 \cdot 01$ have been used in arriving at conclusions.

IV. Experimental Results.

A. *Body Weights.*

Table No. 2, which has been divided into three parts, gives in Section A the average body weights of the Bonemeal and Control calves at 30 day intervals from birth until 480 days of age.

(1) "*Tolly calf*": A castrated bull calf. "Tollies" are castrated bull calves up to the age of 18 months.

TABLE No. 2.

LIVE WEIGHTS.—*Bonemeal Group compared with Control Group.*

Group. A.	No. of Animals per Group.	Average Weight per Calf per Group at different ages, given in days.														
		30	60	90	120	150	180	210	240	270	330	360	390	420	450	480
Bonemeal	9	110	153	204	242	273	284	288	296	306	342	367	416	473	529	561
Control	7	108	149	199	229	265	279	280	291	293	331	338	375	439	496	545
Actual Difference	—	+ 2	+ 4	+ 5	+13	+ 8	+ 5	+ 8	+ 5	+13	+ 11	+ 29	+ 41(5)	+ 34	+ 33	+ 16
Percentage Difference	—	+1·85	+2·68	+2·51	+5·68	+3·02	+1·79	+2·86	+1·72	+4·44	+3·32	+8·58	+**10·93**	+7·74	+6·65	+29·4

Group. B.	No. of Animals per Group.	Average Weight per Calf per Group at different ages, given in days.														
		30	60	90	120	150	180	210	240	270	330	360	390	420	450	480
Bonemeal	4	104	143	192	229	257	269	273	285	298	338	361	415	466	519	541
Control	3	102	140	185	221	245	261	261	275	277	309	316	357	413	466	508
Actual Difference	—	+ 2	+ 3	+ 7	+ 8	+12	+ 8	+12	+10	+21	+ 29	+ 45	+ 58	+ 53	+ 53	+ 33
Percentage Difference	—	+1·96	+2·14	+3·78	+3·62	+4·90	+3·07	+4·60	+3·64	+7·58	+ 9·39	+14·24	+16·25	+12·83	+11·37	+ 6·50

Group. C.	No. of Animals per Group.	Average Weight per Calf per Group at different ages, given in days.														
		30	60	90	120	150	180	210	240	270	330	360	390	420	450	480
Bonemeal	5	115	161	214	252	286	296	300	308	310	345	372	416	478	538	578
Control	4	113	156	209	244	280	293	295	303	306	348	354	388	458	518	573
Actual Difference	—	+ 2	+ 5	+ 5	+ 8	+ 6	+ 3	+ 5	+ 5	+ 4	— 3	+ 18	+ 28	+ 20	+ 20	+ 5
Percentage Difference	—	+1·77	+3·21	+2·39	+3·28	+2·14	+1·02	+1·69	+1·65	+1·31	— 0·86	+ 5·08	+ 7·22	+ 4·37	+ 3·86	+ 0·87

N.B.—(5) Indicates significance at P = 0·05.
(1) Indicates significance at P = 0·01.

The original experimental programme covered the period from birth to weaning. At this latter stage, it was decided to continue the test to 480 days of age, but in the meantime a number of calves had not been weighed when 300 days old. The weight column for this age has therefore been excluded from the table.

The actual differences between the average weights of the bonemeal and control calves are given on the 5th horizontal line. In horizontal line No. 6 these differences are given as percentages of the control (basal) weights. Significant differences are printed in heavy type and to show the degree of significance either a 5 or a 1 has been placed in brackets above and to the right of such significant differences.

Section B gives similar data as Section A, for the heifer calves in the two experimental groups and Section C for the tolly calves. The three ages of importance in the test were, firstly that at 30 days, when the bone meal group was placed upon its phosphorus supplement; secondly at 270 days, when both groups were weaned, and the control group was placed upon a bonemeal ration, equal to that of the bonemeal group; and thirdly at 480 days, when both groups had received an equal phosphorus supplement for 7 months, and when the test was terminated.

Section A of Table No. 2 shows that at none of these ages did there exist any significant difference in body weight between the experimental groups. Only one significant difference is seen in the table, i.e. in Section A at 390 days. Since, however, this significant difference is preceded and succeeded by a series of insignificant differences, its appearance is most difficult to explain and does not appear to be of any biological importance.

It appears warranted to conclude therefore that as far as body-weight is concerned, the feeding of bonemeal to calves prior to weaning was without effect.

Sections B and C of Table 2, show that there is no significant differences between bonemeal and control heifers, and between bonemeal and control tollies. There is, however, a very material difference between the two sexes at the same age. This aspect will be dealt with in a separate article.

B. *Pica.*

Table No. 3 gives the results of the pica tests for each individual animal and for each group, at fortnightly intervals, up to 480 days of age. Three symbols are used:—

(*a*) A minus sign, –, denotes a non-craver.

(*b*) A cross, ×, denotes a rotten bone craver.

(*c*) A plain circle, ○, denotes a sweet bone craver.

The table is divided into two parts. The upper half gives the pica results of the bonemeal group, the lower half those for the control group. The thick vertical line at 30 days in the upper half denotes the beginning of bonemeal supplementation to the bonemeal

group and a similar line in the lower half at 271 days, the beginning of bonemeal feeding to the control group. In the horizontal line below each group, are found, the group pica totals for all age points. In the last 3 vertical columns are found, for each animal and for each group the total number of occassions on which rotten and sweet bones were eaten and the total craving per group.

The table needs no explanation. The calves of the bonemeal group show a negligible degree of craving. Five out of the nine animals never craved at all and of the four that did, the worst (No. 6777), chewed rotten bones only on one occasion and sweet bones only on 3 occasions, during 34 consecutive tests. The total craving for the group amounts to 9 out of a possible number of 306, i.e. 2·94 per cent.

The total craving for the control group amounts to 88 out of a possible 238, i.e. 36·97 per cent. This is 12½ times as much as in the bonemeal group. This high degree of pica is, however, not common to all the calves in the control group. It will be noticed that although no calves were non-cravers throughout the course of the experiment, two were practically so. The rest were all marked cravers. As a group it will be noticed that the degree of pica increased up to the time when all the calves were weaned, i.e. as long as the animals received no bonemeal. From then onwards the pica in the control group, dropped steadily until, for the last 3 tests, only one calf showed craving.

From the data given in Table No. 2 one is justified to conclude—

(*a*) that in the bonemeal group, the phosphorus supplement prevented craving almost entirely;

(*b*) that in the control group, until weaning age, the degree of pica, indicative of aphosphorosis, increased steadily;

(*c*) that the bonemeal supplement given to the calves of the control group, from weaning onwards, reduced the degree of pica to a negligible amount by the end of the test.

C. *Body Measurements.*

Because all the calves were not measured at 30 days of age, Table No. 4 does not include data for that age. It gives the average body measurements in cm. of the calves in both groups, for the ages of 60, 270 and 480 days. As in Table No. 2, the actual and percentage differences (in terms of the control measurements) are given for each age, and significant differences are printed in thick type, with the degree of significance given within brackets above and to the right of the significant differences.

It will be noticed from Table No. 4 that no significant differences were found between the bonemeal and control groups, in the body measurements, at any of the three ages given. In fact, although the calves were measured every 30 days, no significant difference between the 2 groups, was found at any stage during the test and it is, therefore, justified to conclude that the bonemeal supplement had no effect upon the body measurements taken.

TABLE NO. 4.

Body Measurements.

Age of Calves in Group.	Bonemeal or Control.	Body Measurements. Length of Body.	Height at Withers.	Height at Hook-bones.	Depth of Chest.	Width of Chest.	Heart Girth.	Length of Rump.	Width between Hook-bones.	Width between Thirls.	Width between Pin Bones.	Length of Head.	Width between Eyes.
60 days............	Bonemeal..	76·5	78·9	83·6	33·9	16·8	89·4	26·7	20·0	23·4	5·5	27·7	11·0
	Control....	76·6	79·9	84·1	33·6	15·6	88·8	26·6	19·5	23·1	5·6	27·7	11·0
Actual difference....	—	0·1	1·0	0·5	0·3	1·2	0·6	0·1	0·5	0·3	0·1	—	—
Percentage Difference	—	0·13	1·25	0·59	0·89	7·69	0·68	0·38	2·56	1·30	1·79	—	—
270 days...........	Bonemeal..	96·1	97·5	102·0	45·0	19·8	115·3	34·6	28·5	29·9	7·5	35·1	13·3
	Control....	94·9	96·9	101·0	44·4	19·2	113·6	34·4	28·2	29·6	7·3	34·3	13·6
Actual difference....	—	+ 1·2	0·6	1·0	0·6	0·6	1·7	+ 0·2	0·3	0·3	0·2	0·8	— 0·3
Percentage difference	—	1·26	0·62	0·99	1·35	3·13	1·50	0·58	1·06	1·01	2·74	2·33	— 2·21
480 days...........	Bonemeal..	113·4	110·0	113·1	53·6	24·5	140·0	39·6	35·2	34·1	8·1	40·9	15·4
	Control....	111·6	108·1	111·7	53·2	23·3	136·9	38·7	34·4	33·8	7·7	40·2	15·7
Actual difference....		1·8	1·9	1·4	0·4	1·2	3·1	0·9	0·8	0·3	0·4	+ 0·7	— 0·3
Percentage difference	—	1·61	1·76	1·25	0·75	5·15	2·26	2·33	2·33	0·89	+ 5·19	1·74	— 1·91

D. *Blood Analysis.*

In Table No. 5 is given the inorganic blood phosphorus in milligrams per 100 c.c. for each calf, at different ages throughout the experiment. Below each group the group totals and averages, at the given age, are found. In the last 2 horizontal columns the actual and percentage differences (in terms of the control figures) are given. Significant differences are shown in the usual way.

For reasons already explained no analysis was conducted when the calves were 270 days old. Unfortunately it is, therefore, impossible to compare the 3 important ages of 30, 270 and 480 days. Nevertheless, the figures in the last horizontal column are most interesting. At the age of 30 days there was no significant difference in inorganic blood phosphorus between the two groups, although the average of the control group is slightly higher than that of the bonemeal group. At 60 days the difference is still not significant but now the bonemeal group is slightly higher than the controls. At 120 days the bonemeal calves show an inorganic blood phosphorus content, which is 21 per cent. higher than that of the controls. At 180 days this significant difference has increased to 45 per cent. It seems safe to assume that at 270 days, when the controls were placed upon a bonemeal ration, the difference would probably have been at least as marked as at 180 days.

The next analysis, at 11 months of age, i.e. after the controls had been on a bonemeal supplement for 2 months shows a distinct drop in the percentage difference between the 2 groups. This difference, however, remains highly significant. At 13 months of age, the inorganic blood phosphorus of the controls was actually significantly higher than that of the bonemeal groups. From this point onwards there was no difference between the two groups.

Comparing the average P_2O_5 figures for the two groups, it will be noticed, that after the high figures of all the experimental calves, up to 60 days (a high inorganic blood phosphorus content is normal for young calves—see Green and Macaskill, 1928) the bonemeal group retains a level of approximately 6 milligrams per 100 c.c. throughout, while in the control group there is a distinct decline until weaning age, but from the time when these calves too were placed upon a phosphorus supplement, the averages show a definite upward trend.

From these figures it is concluded, that the bonemeal group of calves received sufficient available P_2O_5 to maintain a constant level of inorganic blood phosphorus, as judged by our analysis. On the other hand the control group of calves probably did not receive sufficient available P_2O_5, and although no conformational evidence of an insufficiency could be detected, the analysis nevertheless revealed a drop in the inorganic blood phosphorus. After the control calves had also been placed upon a bonemeal supplement, their inorganic blood phosphorus, rose to a level, equivalent to that of the bonemeal calves.

In addition it must be emphasized that the average values of 5·52, 4·33 and 5·10 milligrams phosphorus per 100 c.c. blood of the control calves respectively at 4, 6 and 11 months of age, are

Table No. 5.

Inorganic Blood Phosphorus.

Group.	No. of Animal.	Inorganic Blood Phosphorus of Calves at Day Ages of.				Inorganic Blood Phosphorus of Calves at the following Approximate Month Ages.				
		30	60	120	180	11	12	13	15	16
Bonemeal	6727	4·5	8·1	6·6	6·0	6·0	6·5	6·0	7·3	6·3
	6730	8·3	8·8	7·3	6·2	6·0	6·0	5·6	8·5	5·0
	6736	—	8·2	6·7	5·7	6·3	6·2	6·2	6·5	6·2
	6740	8·2	7·8	6·0	5·9	6·4	5·0	5·5	4·4	5·7
	6741	7·0	—	6·9	—	7·2	5·9	6·2	7·3	6·2
	6773	—	9·4	5·6	6·5	7·1	6·5	6·2	8·7	5·9
	6777	8·7	8·2	5·8	6·5	5·7	6·5	6·1	7·7	5·3
	6789	—	8·8	7·9	6·8	8·2	7·2	6·0	6·5	5·8
	6790	8·7	8·0	7·3	6·6	7·5	6·4	6·3	8·2	5·7
Totals	—	45·4	67·3	60·1	50·2	60·4	56·2	54·1	65·1	52·1
Averages	—	7·57	8·41	6·68	6·28	6·71	6·24	6·01	7·23	5·79
Control	6714	7·7	8·7	5·6	3·8	5·5	6·2	5·7	7·7	5·5
	6728	5·0	8·3	5·7	5·9	5·7	6·2	6·8	7·3	5·6
	6742	9·7	8·2	5·7	4·2	5·0	5·3	6·7	7·1	5·2
	6743	9·9	7·6	4·6	3·7	5·3	7·7	7·0	6·9	6·6
	6748	—	9·0	4·3	4·1	5·2	6·1	6·7	7·1	5·7
	6749	8·2	—	5·8	3·9	5·2	5·7	6·9	6·2	5·9
	6768	8·2	8·2	5·9	4·7	3·8	6·9	6·6	9·0	5·8
Totals		48·7	50·0	37·6	30·3	35·7	44·1	46·4	51·3	40·3
Averages		8·17	8·33	5·52	4·33	5·10	6·30	6·63	7·33	5·76
Actual differences		− 0·60	+ 0·08	+ 1·16	+ 1·95	+ 1·61	− 0·06	− 0·62	− 0·10	+ 0·03
Percentage differences	—	− 7·34	+ 0·96	**+21·01**(1)	**+45·03**(1)	**+31·57**(1)	− 0·95	**− 9·35**(1)	− 1·36	+ 0·52

(5) Indicates significance at P = 0·05.
(1) Indicates significance at P = 0·01.

indicative of aphosphorosis. The value 4·44 especially, is only just above the minimum accepted as sufficient for mature cattle, and is therefore rather low for 6 months old calves. This provides evidence, that the control calves suffered from a phosphorus deficiency, which however, was not manifested conformationally.

E. *Bone Histology.*

The histological examination of the costo-chondral junctions showed good calcification with long and closely knit primary trabeculae, both in the rib-resections taken at 65 and at 180 days of age. Up to 6 months of age, therefore, the skeletons of the calves examined were found to be normal in terms of phosphorus. It is unfortunate that no histological examinations were done on resections from calves at 9 months of age.

In the resections taken in June 1937 (180 days old) the costo-chondral junctions of calves Nos. 6714 (control group) and 6727 (bonemeal group) revealed the presence of a fibrosis, probably osteodystrophic in nature, which is, however, as far as we know, not associated with aphosphorosis.

V. Discussion.

Note.—In the discussion of the experimental results the word "function" is used in such phrases as "functional efficiency", "functionally subnormal", "functional abnormalities", etc. The reader may interpret the word "function" as the sum total of an animal's physiological manifestations. In the present discussion such an interpretation is not intended. The actual functions referred to are clearly described in paragraphs (*b*) (1) and (*b*) (2), below.

The results of the experiment show, that in terms of the experimental criteria employed:—

(*a*) *The bonemeal supplement had no effect upon growth and development, i.e. upon external conformation*: At no time during the course of the investigation, either before or after weaning, were significant differences found between the average body weights or body measurements of the two groups.

(*b*) *The bonemeal supplement improved functional efficiency*: The bonemeal calves, functioned on a higher plane of efficiency than did the control calves, from the time they were placed upon a phosphorus supplement, until the control calves too received the same supplement.

(1) In comparison with the controls, they exhibited practically no osteophagia, and showed a higher and much more constant level of inorganic blood phosphorus up to weaning age.

(2) From the age of weaning onwards, when the control calves also received bonemeal, the manifestations of a phosphorus deficiency exhibited by them abated and finally disappeared. At the conclusion of the experiment, the control calves were, in terms of the experimental criteria, in every way comparable to the bonemeal calves.

The experimental results show up an apparent anomaly. At weaning age the control calves, although conformationally normal, were functionally subnormal. How can subnormal function maintain normal conformational development? Theiler, Du Toit and Malan (1937) conducted mineral metabolism investigations on pigs. They found that a group which received an adequate ration, except for a deficiency in phosphorus, showed as rapid a weight increase, as did the group on a completely adequate ration, for the first 5 months of the experiment, i.e. up to a live weight of approximately 140 lb. This normal conformational development took place notwithstanding the fact, that 4 months after the commencement of the test, the inorganic blood phosphorus of the group on the deficient ration averaged only 3·65 milligrams P_2O_5 per 100 c.c. as compared with 8·9 milligrams per 100 c.c. for the group on a complete ration.

Up to a point, therefore, the deficient skeleton was able to support normal increase in body weight. After this limital point had been passed, the phosphorus deficient group showed a rapid decline in the rate of bodyweight increase in comparison to the group which received phosphates.

It would appear as if, *up to a point*, the skeletons of growing animals are able to do with less than their normal mineral requirements, and nevertheless function sufficiently well to support normal increase in body weight and size.

This idea explains the apparent anomaly encountered in the experiment under discussion. The control calves, although receiving a phosphorus deficient diet, had not reached the "critical point". at weaning age, and therefore, showed no externally determinable symptoms of the deficiency, which, when removed by subsequent bonemeal feeding, allowed the animals to build up the functional abnormalities back to normal by the time they were 480 days old.

It must be remembered, however, that the calves of both experimental groups were born out of bonemeal fed cows, and received, through the milk of their dams, quite an appreciable amount of available phosphates, up to weaning age.

On the average each calf of the bonemeal group received 1,251 lb. of milk during the 210 day period over which the production of its dam was recorded. The control calves each received on the average 1,288 lb. of milk. The difference of 37 lb. per calf between the 2 groups is insignificant. Even if the difference was significant it would be in favour of the control group.

Groenewald (1934) gives an average of 0·221 per cent. of total P_2O_5 in bovine milk. Applying this figure to the average milk intake of all the calves born out of bonemeal fed cows, that is to 1,267 lb., we find that each calf received on the average 1,258 grammes of total P_2O_5 in 210 days or 6·00 grammes per head per day.

This amount, together with whatever available P_2O_5 they obtained through their grazing, was apparently sufficient for the control group to maintain normal conformational development, and to maintain functional efficiency, above the "critical point" up to 270 days of age.

The two groups of calves ran together in the same paddocks and received the same amounts of milk. Therefore the fact that the bonemeal calves were both conformationally and functionally normal, while the control calves, though conformationally normal, were functionally subnormal, can only be attributed to the extra phosphates which the former group received from their supplement. The phosphorus deficiency in the controls was not sufficiently acute to manifest itself conformationally.

So far we have compared groups of calves born of bonemeal fed cows, and which received the same average amount of milk from their dams. Let us next compare the control group of calves, in the test under discussion, with a group of otherwise comparable animals, but born out of non-bonemeal fed cows. To distinguish the two groups, the former will be referred to as the "bonemeal control group" and the latter as the "straight control group". The data for this latter group have been obtained from the records of other experiments, conducted at "Armoedsvlakte". No heifer calves and only 3 tollies were found, which were fully comparable to the "bonemeal control" calves, and therefore Table No. 6 gives the data of these 3 tollies against that of the 4 tollies in the "bonemeal control" group.(1)

The table is divided into sections. In Section I, the weights for each of the "bonemeal control" calves are given monthly from birth until the end of the test. Below these weight figures, are found the average daily amounts of milk each calf obtained from its dam, during the preceding month. Because milk recording at "Armoedsvlakte" takes place only for 210 days, only 7 milk averages are given.

Section II gives similar data for the "straight control" group. In Section III, the actual differences in body weight and in milk supply between the two groups, are given in the first and third, and in the second and fourth horizontal lines; these differences are given as percentages of the "straight control" figures. Significant percentage differences are shown in thick print and the degree of significance indicated in the usual manner.

Section No. III of Table No. 6, shows that at birth the "bonemeal control" calves, weighed on the average 7 lb. more than the "straight control" calves. This difference is not significant; in fact Du Toit and Bisschop (1929) found, when working with a much larger number of grade Redpoll calves, that the difference

(1) *Explanatory Note by the Statistician.*—The small number of animals in each group, is a serious objection, since the co-efficient of variation tends to be higher than that of large groups. The statistical test of significance requires that the differences between small groups have, in any case, to be much greater than between large groups, but when coupled with a high coefficient of variation these differences have to be even larger. The lack of significance in the very large percentage differences in the last horizontal line of Section III of Table No. 6 may be explained on this basis. Small groups therefore lead to fewer significant differences, but it should be noted that the power of the statistical analysis is such that the significant differences that are obtained, may be accepted with the same measure of confidence, attributed to differences between larger groups.

between calves of bonemeal fed and non-bonemeal fed cows, at birth to be only 1·9 lb., the respective average weights being 69·2 lb. and 67·3 lb. They further found that where similar figures were worked out for four different breeds, the respective average birth weight for calves of bonemeal fed cows was 68·8 lb., and for calves of non-bonemeal fed cows 68·1 lb.—a difference of only 0·7 lb.

In the table under discussion, the insignificant difference in live weight at birth soon became significant and increased until at weaning age the "bonemeal control" calves weighed 53 lb, or 20·95 per cent. more than the "straight control" calves. At 16 months of age this difference had increased to 159 lb., or 38·41 per cent.

In terms of live weight, therefore, the development of the "straight control" calves was subnormal when compared with the development of the "bonemeal control" calves.

It may, however, be argued that the ever-increasing differences in live weight between the two groups, were the result of the initial difference at birth. In Section No. IV, all the weights have been corrected for the birth difference. [According to R. A. Fishers (1936) "Analysis of co-variance technique".] If now the actual difference in live weight between the 2 groups were but manifestations of the difference which existed at birth, all significant differences should disappear in Section IV. This does not happen, and it is permitted to conclude, that the difference in weight at birth was not the cause of the marked differences later on in life.

What then caused the marked difference in body weights between the two groups?

In Section No. V of Table No. 6 the weights of the two groups of calves, have been corrected for difference in average milk intake (according to R. A. Fishers' 1936 "Analysis of co-variance technique"), and it will be noticed that *all significant differences now fall away*. This proves, that the differences in live weights between the "bonemeal control" and "straight control" groups were caused by the difference in the amounts of milk, which they got from their dams.

Green (1926), Becker, Eckles and Palmer (1927), Groenewald (1934) and others, have shown that, with the exception of fat, the percentage of which is inversely proportional to the amount of milk produced, there is no significant difference in the chemical analysis of the milk of phosphorus fed and non-phosphorus fed cows. The difference in body weights between the "bonemeal control" and "straight control" calves must therefore be attributed to the straight quantitative, and not also to the qualitative difference of their milk intake.

The average milk intake of the four "bonemeal control" tollies which appear in Table No. 6, amounted to 1,278 lb. in 210 days. The three "straight control" calves each received 782 lb. during the same period: a difference of 496 lb., or 63·4 per cent. of the average "straight control" figure.

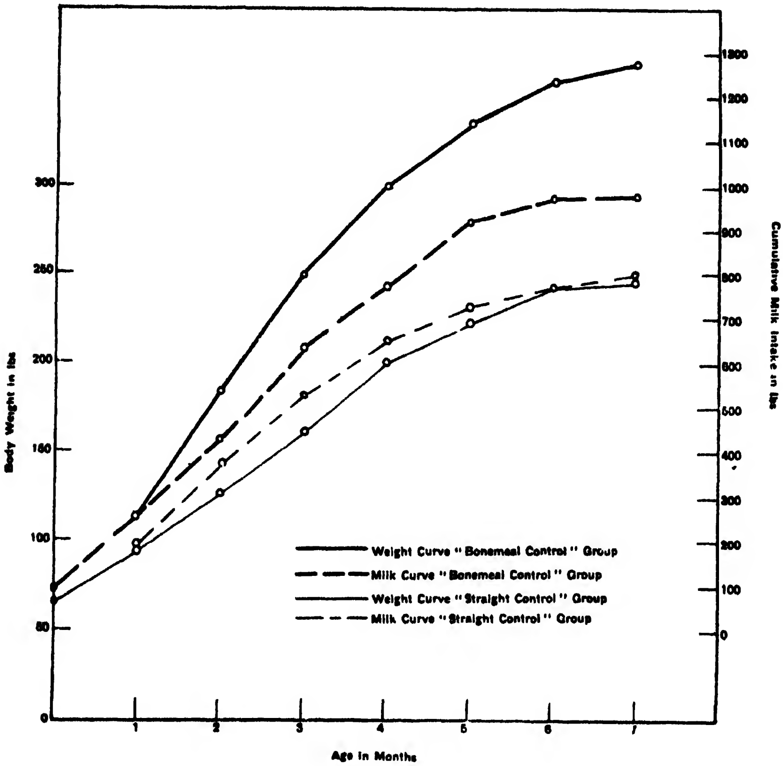
We have seen that both the " bonemeal " and bonemeal control " calves were born out of bonemeal fed cows. On the average they all got 1,267 lb. of milk in 210 days. This milk intake enabled them to develop normally and equally in terms of body weights and body measurements. Functionally, however, the " bonemeal control " calves were subnormal until they also received a bonemeal supplement, but their functional efficiency never fell to below the " critical point ". In them an intake of 1,267 lb. of milk over 210 days, prevented functional efficiency from becoming so depressed as to produce conformational effects. In the " straight control " calves, the very low milk intake depressed functional efficiency to below the " critical point " during the pre-weaning period, and resulted conformationally in subnormal body weights very early in life. In the comparison between " bonemeal " and " bonemeal control " calves, the difference in functional efficiency was definitely due to the difference in P_2O_5 intake. As shown, this amounted to about 12 grammes of available P_2O_5 per calf per day in favour of the " bonemeal " group. In the comparison of the " bonemeal control " and " straight control " groups, the subnormal body weights of the latter were not due only to a lesser P_2O_5 intake. Applying composition figures given by Davis (1936) and Groenewald (1934), the 496 lb. milk intake difference betwen the two groups, amounted to a difference per calf per day of 2·3 grammes of P_2O_5, 35 grammes of protein and 135 grammes of carbohydrates, apart from differences in other food constituents.

Graph No. 1 gives curves for the average body weights of the two groups from birth to 7 months of age, and for the average accumulative amounts of milk the calves received from month to month over the same period.

The graph illustrates quite definitely how the weight curves follow the milk curves.

If we make a closer study of Sections I and II of Table No. 6 we find that in general, weight reacts to milk intake to a marked degree. For example, calf No. 6714 weighed 66 lb. at birth, while calf No. 6742 weighed 81 lb. For the first 3 months the latter received more milk than the former and more than maintained its advantage in weight. From the fourth to the seventh month the milk intake of No. 6742 dropped to below that of No. 6714, the live weights of which progressively approached those of No. 6742, and passed them from the 7th month, onwards. In the table this same phenomenon occurs between any two animals which commence their life with different amounts of milk, and where the one, which received the lesser amount to start off with, receives the larger amount during the latter part of the pre-weaning period. When in the comparison of any two animals in the Table, the milk supply of one remains higher than that of the other throughout the pre-weaning period, the body weights will show the same relationship. A comparison for example between Nos. 6714 and 6748, which had similar birth weights, illustrates the latter point.

GRAPH NO. I



SUMMARY.

The results are presented of an experiment to ascertain whether a regular supplement of bonemeal given to calves up to weaning age would be beneficial to their growth and development, in comparison with a comparable group of calves which received no bonemeal up to weaning. From weaning onwards, both groups received the same supplement. The average milk intake for the groups was the same. All the calves were weighed and measured every 30 days from birth until the conclusion of the test at 480 days of age. They were tested for osteophagia at fortnightly intervals and a blood-sample for inorganic blood phosphorus determination was collected from each calf at approximately monthly intervals.

The results are compared with data which were collected on comparable calves, not included in the experiment. They were born out of cows which received no bonemeal supplement and therefore gave to their calves, on the average 496 lb. less milk than did the bonemeal fed dams of the calves in the experiment. This comparison demonstrates the effect of a decreased milk intake upon growth and development.

Conclusions.

(*a*) Under open range conditions in Bechuanaland, the quantitave milk intake of a calf during its pre-weaning period of life, determines its body or conformational development.

(*b*) In general, bonemeal fed cows, living under open range conditions in Bechuanaland supply their calves with sufficient milk for normal development up to weaning age.

(*c*) In Bechuanaland, cows which receive no phosphorus supplement, do not as a rule supply their calves with sufficient milk for normal development up to weaning age.

(*d*) Although the milk supply of cows receiving a phosphorus supplement is as a rule sufficient for the normal conformational development of their calves, such calves nevertheless are functionally subnormal as indicated by low inorganic phosphorus in their blood.

(*e*) This phosphorus deficiency can be corrected by feeding phosphates prior to weaning.

(*f*) Such a supplement prior to weaning, however, is not necessary, if the calves receive the necessary phosphates from weaning onwards.

(*g*) Up to a point, skeletons of growing calves are able to do with less than their normal mineral requirements, and nevertheless function sufficiently well to support normal increase in body weight and body size.

(*h*) The "critical point" is not reached prior to weaning age in the calves of bonemeal fed cows, and therefore such calves, although functionally subnormal, are conformationally normal.

(*i*) In the calves of non-phosphorus fed cows this "critical point" is reached prior to weaning. Such calves are both functionally and conformationally subnormal.

References.

BECKER, ECKLES AND PALMER (1927). *Jnl. Dairy Science*, Vol. 10, p. 169. Quoted by Davis (1936), p. 177.

DAVIES, W. L. (1936). The chemistry of milk. Chapman and Hill.

DU TOIT, P. J. AND BISSCHOP, J. H. R. (1929). The breeding of cattle on phosphorus deficient veld. *15th Annual Report Director Vet. Research, Union of S. Africa*, pp. 1059-1166.

DU TOIT, P. J. *et al* (1932). A study of the mineral contents and feeding value of natural pastures in the Union of South Africa (1st Report). *18th Report of the Director of Vet. Services and Animal Industry*, pp. 525-577.

DU TOIT, P. J. *et al* (1935). A study of the mineral contents and feeding value of natural pastures in the Union of South Africa (3rd Report). *Onderstepoort Jnl. of Vet Sc. and An. Industry*, Vol. 5, No. 1, pp. 201-214.

DU TOIT, P. J. *et al* (1938). A study of the mineral contents and feeding value of natural pastures in the Union of South Africa (6th Report). In the press.

FISHER, R. A. (1936). Statistical methods for research workers (6th Edition). Oliver and Boyd, Edinburgh.

GREEN, H. H. (1926). Unpublished data.

GREEN, H. H. AND MACASKILL (1928). Studies in mineral metabolism No. 6. Composition of the blood of cow and calf in respect to mineral constituents. *Jnl. Agr. Sc.*, Vol. 18, No. 3, pp. 384.

GROENEWALD, J. W. (1934). The influence of rations low in certain minerals on the composition of the blood and milk of cows and on the blood of their progeny. *Onderstepoort Jnl.*, Vol. 4, No. 1, pp. 93-165.

HENRICI, M. (1928). The phosphorus content of the grasses of Bechuanaland in the course of their development. *13th and 14th Reports of the Director of Vet. Education and Research, Union of South Africa*, pp. 1077-1208.

THEILER, A., DU TOIT, P. J. AND MALAN, A. I. (1937). Studies in mineral metabolishm No. 38. Calcium and phosphorus in the nutrition of growing pigs. *Onderstepoort Jnl.*, Vol. 9, No. 1, pp. 127-164.

THEILER, A., GREEN, H. H. AND DU TOIT, P. J. (1924). Phosphorus in the livestock industry. *Jnl. Dept. Agr., Union of S. Africa*, pp. 460-504.

Section V.

Toxicology.

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Alkali Poisoning.

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I. Weak Alkalis (Sodium Bicarbonate and Sodium Carbonate).

Kobert (1906) states that sodium bicarbonate raises the alkalinity of the tissue fluids and enhances the oxidation processes in the body. Ingestion of excessive quantities of sodium bicarbonate causes dilatation of the stomach, anorexia and anaemia.

Fröhner (1919) reports that a cow which had received 800-900 gm. of sodium carbonate aborted, groaned and bellowed, and had to be slaughtered, and that 10-15 gm. of potassium carbonate is fatal for the dog (heart failure and collapse). Dogs fed daily with 15 gm. of sodium bicarbonate for weeks showed vomiting, diarrhoea and loss in condition (Fröhner, 1919). He also states that two horses which ate sodium carbonate from bags took ill with severe colic, profuse diarrhoea, continuous coma, stomatitis and swelling of the lips.

Reid (1921) describes sodium carbonate poisoning in sheep due to the drinking of effluents from dairy factories. The symptoms were staggering gait and hurried respirations. The animals soon lay down and died. The following were the post mortem appearances: Hyperaemia of the subcutaneous tissues, imperfect clotting of the blood, hyperaemia of the small intestines with subserous haemorrhages, slight congestion of the liver, in very acute cases the abomasum was filled with dark, blood-stained, fluid contents, intense hyperaemia of abomasal mucosa with haemorrhages.

Heller and Larwood (1930) conducted experiments upon rats and other small animals with single salts and also mixtures of calcium chloride, calcium sulphate, sodium bicarbonate, and magnesium sulphate. Drinking water containing 20,000 parts of sodium carbonate per million parts (i.e. a 2 per cent. aqueous solution) was found to be decidedly deleterious and reproduction was interfered with at quite low levels. Sodium bicarbonate proved to be less injurious. In subsequent experiments upon rats Heller (1932 and 1933) established that drinking water containing 1 per cent. (10,000 p.p.m.) of sodium carbonate resulted in unsatisfactory growth of the offspring, while a 1·9 per cent. solution caused a rough coat, red eyes, and diarrhoea in mature rats and a very high

mortality in their young. A 1·5 per cent. solution of sodium bicarbonate caused undersized adults and impeded growth in their offspring, while there were unsatisfactory growth and appearance with 2 per cent. solutions. Experimenting with 0·5 and 1·0 per cent. aqueous solutions of caustic soda (NaOH) he found practically normal growth with the former solution, the latter causing retarded growth, "dirty animals", marked nervousness, sore eyes and diarrhoea.

Linton and Wilson (1933) refer to sodium bicarbonate poisoning in pigs due to the feeding of "Flour sweepings" containing 33·7 per cent. of this alkali. The symptoms seen were excessive thirst, animals appeared to be in pain, stood with their heads lowered and oblivious to their surroundings and staggered on their front legs. Only one pig died after the sweepings were removed and milk given. Autopsy revealed inflammation of the stomach. Linton furthermore describes alkali poisoning in pigs through accidental discharging into their milk of the alkali used for cleansing the milk utensils.

Some time ago the author received for analysis a certain proprietary "scouring powder", which was being sold as a cleansing agent for *kitchen utensils.* Pigs had died after their troughs had been scoured with it. Four grams of this strongly alkaline powder caused death in rabbits within a few hours. This is one of the many cases in which a virulent poison is labelled non-poisonous.

Witter (1936) added sodium bicarbonate to the drinking water of chicks and found that: "(1) Sodium bicarbonate given in the drinking water in the usual dosage (¼ lb. to 5 gallons or 0·6 per cent. solution) caused chicks to drink more water than normal and produce moist droppings. Chicks two weeks old developed pale and swollen kidneys from this dosage, but chicks three weeks old and older were not noticeably injured. (2) A double dose (1·2 per cent. solution) of soda caused chicks to drink more water than those fed the 0·6 per cent. solution and produced watery droppings. Chicks two to eight weeks old were seriously injured by this dosage within one to three days and deaths occurred within this time. (3) Two and four-tenths per cent. solution of soda reduced water consumption below normal for chicks under four weeks of age. The injurious effects of this dosage were noted within a day and deaths occurred within three days. (4) Mature cockerels were injured with a 2·4 per cent. solution of soda, but were not affected by a 1·2 per cent. solution. It was apparent throughout the project that the younger the chicks the more susceptible they were to soda injury. (5) Kidneys from chicks affected by feeding soda became pale, swollen and engorged with urates. The kidney tubules showed degenerative and exudative changes indicating severe injury. (6) Chicks affected by feeding soda showed an increase in kidney weight, and an increase of approximately four times in uric acid per gram of kidney and in uric acid in the blood". He found the injury caused by feeding sodium bicarbonate to chicks similar to the pathology and blood chemistry changes present in visceral gout. Two weeks old chicks consumed from 45 to 113 c.c. of "normal water" daily.

Carbonates, bicarbonates and caustic soda tend to cause alkalinity of the gastrointestinal juices and of the blood and tissue fluids (change in pH), consequently the continued drinking of alkaline waters and the constant taking of alkaline waters and the constant taking of alkaline powders will lead to serious digestive and other disturbances. Carbonates are more harmful than bicarbonates.

Cope (1936) draws attention to the fact that the constant use of alkaline stomach powders by individuals suffering from chronic gastric hyperacidity may have serious consequences. These powders generally contain calcium carbonate, magnesium carbonate, bismuth oxycarbonate, and sodium bicarbonate. He states that the following symptoms may arise in the course of time when alkaline powders are taken over long periods: Loss of appetite; slight vomiting; irritability; thoughtlessness; unreasonableness; depression; vague headaches and muscle pains; red and inflamed conjunctiva (sore eyes); "vision may be slightly blurred, but is as a rule not interfered with"; in some cases the patient himself complains about increasing impairment of mental efficiency; the skeletal muscles are sometimes abnormally excitable to direct percussion; polyuria is usual, but sometimes the urine volume is reduced below normal; the urine is mostly alkaline and always contains albumin; finally there may be coma. "The rapidity with which these successive stages are passed through varies considerably"—from a few days to weeks. Cope states that "very characteristic of all conditions of this type in which the body contains excess alkali is the extremely low concentration of chloride in the urine. This may be very simply demonstrated by the addition of nitric acid and a small quantity of silver nitrate solution." According to Cope the following points are of importance in the diagnosis of alkali poisoning: (1) The symptoms. (2) The presence of albumen in the urine. (3) Low concentration of chloride in the urine. (4) "An alkaline reaction of the urine with effervescence on the addition of acid is of no diagnostic value as it is frequently found in persons taking considerable quantities of alkali in the complete absence of all toxic symptoms." (5) Blood analysis: (*a*) "Estimation of plasma carbonate or alkali reserve is the most certain of interpretation"—a rise above 80 volumes per cent. is practically diagnostic of alkali poisoning. (*b*) "It would seem to be a safe general rule, however, to regard all suspected cases showing a rise in blood urea of about 80 mg. per cent. as cases of alkali poisoning, unless an independent cause for the high value can be definitely found." As treatment he recommends: (1) Removal of the alkaline powders. Alkali sensitive individuals should take di-sodium phosphate or perhaps hydrated magnesium silicate. (2) Administration of sodium chloride *per rectum* facilitates the excretion of bicarbonate. (3) Ammonium chloride can be given as an acidifying agent, but it must be used with care as in all cases of alkali poisoning the kidneys are severely damaged and acidosis may result. It is essential that the urine be examined at frequent intervals.

In experiments upon themselves Joos and Mecke (1934) found that after the administration of sodium bicarbonate there is (1) a marked increase in the sodium content of the blood serum, (2) a

slow fall in the magnesium content, (3) a pronounced and sudden fall in the calcium content, (4) a quick fall in the chloride content, and (5) a slow fall in the phosphorus content of the blood serum.

Little (1937) describes a simple method of estimating the alkaline constituents of washing powders and washing solutions containing mixed alkalies.

The taking of too much alkaline stomach powders has resulted in a serious state of alkalosis (Davies, 1939).

II. Caustic Alkalis (Caustic Soda and Caustic Potash).

A. *Onderstepoort Experiments.*

Some time ago a specimen consisting of a fairly dark-brown, semi-coagulated, jelly-like fluid was submitted by the Government Veterinary Officer, Greytown, Natal, to Onderstepoort for analysis. It was stated that it was a specimen of milk which had turned this colour when placed on the stove to boil. The specimen was submitted in an air-tight fruit jar and was about eight days old when examined. It emitted a very pronounced alkaline odour, was slimy and jellylike, and yielded a marked alkaline reaction with litmus paper. It was impossible to ascertain the degree of alkalinity by means of titration as the filtrate was dark-brown in colour and could not be decolorised with charcoal. It was then decided to determine the sodium content of the specimen. This was found to be far in excess of the sodium content of normal milk.

The following rabbits were drenched by means of a stomach tube with the above specimen:—

Rabbit A (2·0 *Kg.*): 100 *c.c. on* 7/7/38.

Within five minutes after drenching pronounced laboured breathing set in, the animal breathing through its mouth. General weakness (paresis) followed soon and the animal was unable to sit up or hold its head up. Muscular twitchings were seen on different parts of the body. The heart-action was very much accelerated and weak in the beginning and became progressively weaker until it was imperceptible a short while before death. The animal died within ten minutes after the development of symptoms. A few minutes before death occurred the animal became completely paralysed, was comatose and lay prostrate with the head drawn backwards. It died with terrific convulsions (jumping about in the cage) of the whole body. These were probably due to asphyxia.

Post mortem appearances: General cyanosis; pronounced hyperaemia of the lungs, liver and kidneys; pronounced dilatation of both heart ventricles; pronounced hyperaemia and sliminess of the gastric mucosa; contents of small intestine very slimy.

Rabbit B (2·0 *Kg.*): 50 *c.c. on* 7/7/38.

Symptoms very similar to those described above set in within ten minutes after drenching. The only difference in the

symptomatology was that there were no muscular twitchings and that the animal died quietly in a state of paralysis and coma. Death occurred approximately twenty minutes after drenching.

Post mortem appearances: Very similar to those described in rabbit A with haemorrhages in the gastric mucosa.

The following three experiments (specimens 1, 2 and 3) were then conducted:—

Specimen (1):

Four grams of granular caustic soda were added to 200 c.c. of fresh cow's milk. A light yellow colour appeared almost immediately. It soon became more intensely yellow and then developed a brownish colour as the temperature of the milk was raised by means of a gas-flame. As the temperature of the milk rose it became dark yellow-brown and when it was boiling it turned a dark reddish-brown colour. Between 50° and 70° the milk jellified and became fluid again before it boiled. It emitted a pronounced alkaline odour.

Specimen (2):

Twenty grams of granular caustic soda were added to 200 c.c. of fresh cow's milk and boiled for two minutes. The milk showed the same changes as those described under (1); the changes in colour took place more rapidly and the colours were more intense. The cooled specimen was then placed in an air-tight fruit jar and was drenched to two rabbits seven weeks later.

Rabbit A (1·9 *Kg.*): 50 *c.c. on* 14/9/38.

The animal developed dyspnoea and apathy within a few minutes after drenching. After having shown loss of appetite for a day it appeared normal again.

Rabbit B (1·95 *Kg.*): 100 *c.c. on* 14/9/38.

Symptoms more pronounced than those described in Rabbit A set in within five minutes after drenching. The animal appeared normal after twenty-four hours.

Specimen (3):

Forty grams of caustic soda were added to 200 c.c. of fresh cow's milk and boiled for two minutes. The results of the addition of caustic soda and boiling were similar but more pronounced than those described under (2). The appearance, colour and odour of this specimen of milk were identical with those of the specimen submitted by the Government Veterinary Officer, Greytown. Two rabbits were drenched with specimen (3) after it had been kept for seven weeks in an air-tight fruit jar.

Rabbit C (1·25 *Kg.*): 50 *c.c. on* 14/9/38.

The only symptoms the animal showed were transitory laboured respiration and loss of appetite.

Rabbit D (1·65 *Kg.*): 100 *c.c. on* 14/9/38.

Laboured respiration set in within a few minutes after drenching. There were violent intestinal movements within thirty minutes. After having shown pronounced apathy, loss of appetite, accelerated heart-action and after having passed dark brown urine for two days the animal appeared normal again.

The slight toxicity of specimens (2) and (3) was thought to be due to the fact that they had stood too long (seven weeks) before being drenched. A further three specimens (4, 5 and 6) of milk were then treated with caustic soda, boiled for two minutes and left standing for eight days in air-tight fruit jars. The changes in the milk were the same as those noted before. On cooling the specimens jellified.

Specimen (4):

Twenty grams of granular caustic soda were added to 200 c.c. of fresh cow's milk. After standing for eight days the specimen was dark brown in colour and had a jellylike appearance and was slimy. It emitted a pronounced alkaline odour and smelt very strongly of ammonia. It was drenched to two rabbits:—

Rabbit E (0·9 *Kg.*): 50 *gm. in a small quantity of water on* 23/9/38.

The animal retched and vomited within a few minutes after drenching, and died within a further two minutes.

Post mortem appearances: Most pronounced hyperaemia of gastric mucosa with partial dissolution (slimy appearance of the mucosa; similar changes in the small intestine; pronounced congestion of the liver; a fair quantity of vomitus in the lungs.

Rabbit F (1·7 *Kg.*): 100 *c.c. with a small quantity of water on* 23/9/38.

Symptoms identical with those described in Rabbit A, which was drenched with the specimen submitted by the Government Veterinary Officer, Greytown, developed within three minutes after drenching. After a further ten minutes the animal retched, vomited and died soon afterwards.

Post mortem appearances: Gastric wall dark reddish-brown in colour and almost completely dissolved (very slimy); mucosa of small intestine dark reddish-brown and slimy (partial dissolution); pronounced hyperaemia of the liver; no vomitus in lungs.

Specimen (5):

Forty grams of caustic soda were added to 200 c.c. of fresh cow's milk. After eight days appearance and odour of this specimen were the same as those of specimen (4). The colour was dark yellowish-brown. The following rabbits were drenched with it:—

Rabbit G (1·25 *Kg.*): 50 *c.c. with a small quantity of water on* 23/9/38.

The symptoms resembled those described in Rabbit F. The animal vomited and died within ten minutes after dosage.

Post mortem appearances: As in Rabbit F, only more pronounced. No vomitus in lungs.

Rabbit H. (1·65 *Kg.*): 100 *c.c. with a small quantity of water on* 23/9/38.

Symptoms as described in Rabbit F appeared within a few minutes and death occurred within ten minutes after drenching. *Post mortem appearances:* As in Rabbit G. The gastric wall was almost completely dissolved and showed a rupture.
Specimen 6:

Sixty grams of caustic soda were added to 200 c.c. of fresh cow's milk. After eight days the colour, appearance and odour (alkaline and ammonia) were identical with those described under specimen (5).

This specimen was not dosed to rabbits as specimens (4) and (5) had yielded positive results.

In considering the toxicity of milk to which caustic alkalis were added it should be realised that there are two different kinds of poisons present, namely, the caustic alkali and decomposition products caused by the action of the alkali on some of the constituents of the milk, especially the protein. That the alkali causes pronounced destruction of the protein in the milk is evident from the pungent ammonia odour which is emitted after a few days. Toxic amines are probably also formed. It appears that the toxicity of caustic alkali milk decreases in the course of time. The ammonia odour which was not pungent after eight days decreased and eventually disappeared.

On an earlier occasion three groups of four rabbits each were drenched with 1·0 (Group I), 2·0 (Group II) and 5 (Group III) per cent. aqueous solutions of caustic soda respectively. The two rabbits (Group I) which received 40 c.c. of a 1·0 per cent. solution developed no symptoms beyond transient loss of appetite. Those two animals of Group I which were drenched with 80 c.c. died within forty hours. In Group II two rabbits received 40 c.c. and the remaining two 80 c.c. of a 2·0 per cent. solution of caustic soda. They died from within twenty to thirty hours after drenching. The two rabbits in Group III which received 40 c.c. of a 5·0 per cent. solution of caustic soda developed symptoms within a few minutes.

One of these rabbits vomited and died within one-and-a-half hours after drenching, whilst the other one lived for approximately thirty hours. The remaining two rabbits of Group III which received 80 c.c. of a 5·0 per cent. caustic soda solution died within three hours.

The symptoms and post mortem appearances closely resembled those described below under II (B). Even with 80 c.c. of a 1·0 per cent. solution of caustic soda there was pronounced dissolution (corrosion) of the gastric mucosa and wall. In one of these cases the gastric wall was almost perforated in two places. The remaining portion of the internal surface of the stomach wall was very slimy and intensely dark (dirty) reddish-brown (alkali haematin). With higher concentrations of caustic soda the lesions were much more pronounced.

B. *Mode of Action, Toxicity and Symptoms of Caustic Alkalis.*

Caustic alkalis dissolve the epithelium and form soluble alkali albuminates when coming into contact with proteins (skin and mucous membranes). The mucous membrane and skin becomes oedematous, transparent, and soapy, and the corroded area becomes necrotic, soft and slimy. If blood vessels have been corroded by the alkali the affected surface may bleed and alkali haematin is formed. In such cases the colour of the affected area may be reddish-brown, black brown (rare) or often bright red (excessive haemorrhage). In the course of time the colour changes to greyish-white—in dilute solutions within 1-2 hours and in concentrated solutions after longer periods.

Acute alkali poisoning facilitates the deposition of calcium in the bone system, hence the blood and tissues are deficient in this element (Rentz, 1933; Renvis, 1935).

Histologic changes in the parenchymatous organs in cases of alkali poisoning have been described by Jankovich, Incze and Fazekas (Fazekas, 1937), but unfortunately the publication is not obtainable in South Africa. Fazekas (1937) drenched different groups of rabbits with 1, 2, 3, 5 and 10 per cent. solutions of caustic soda, the animals receiving 0·5, 0·75, 1·25, 1·5, 2·25 and 2·5 gm. NaOH after one day starvation. The following changes were noted in the blood chemistry of the animals:—(1) A disturbance of the glucose metabolism in the form of hyperglycaemia. (2) A pronounced increase in the inorganic phosphorus content of the total blood. (3) A decrease in the serum calcium. (4) A moderate decrease in serum chlorine. (5) A slight increase in serum sodium. (6) Definite acidosis in the form of a decrease in serum alkali reserve.

The degree of corrosion caused by caustic alkali depends upon: (1) Their concentration. (2) The quantity swallowed. (3) Length of period of action. (4) Quantity of food present in the stomach and intestine. (5) The resistance of the tissues ~~concerned~~. Solutions of caustic alkalis under 1·0 per cent. do not as a rule cause fatal corrosion but mostly only inflammation of the mucous membranes. More concentrated solutions and especially solid pieces of alkali

frequently cause severe corrosion and even perforation of mucous membranes and of the stomach wall. When solutions of caustic alkali are thrown in the face it may be aspirated and fatal pneumonia may result.

Balasz (1934) states that 2,134 cases of alkali poisoning were treated in the Budapest hospital from 1924-1933. Most of these cases were suicidal. He gives a detailed description of the symptoms in alkali poisoning.

The following symptoms are seen in cases where solutions of caustic alkalis of 10 per cent. and higher concentrations are swallowed:—The lips are swollen in almost all cases of alkali poisoning. This is differential-diagnostically of great value in comparison with acid poisoning. Very severe pain in all the organs with which the solution comes into contact. Pain is especially experienced in the sternal region and in the back due to oesophagitis and perioesophagitis. On the first day there are stomach pains in the cardiac and epigastric regions. There are hiccoughs and vomiting. The vomitus may be mucoid, slippery and reddish-brown or almost black with alkali haematin or it may be bright red when excessve bleeding occurs. When necrotic material is present it has a very bad odour. There is difficulty in swallowing which may be due to pain, spasms, an acute, transitory, reflex paralysis, or paralysis due to direct alkali action on the soft palate or oesophageal musculature, swelling of the oesophageal mucous membrane, mechanical blocking of the oesophagus by necrotic mucous membranes, food, or due to fibrosis of the muscles of deglutition. The respiration is accelerated and in bad cases the pulse is slow and small (shock) in the beginning, but eventually it becomes accelerated in most cases. Prognostically a fast pulse is unfavourable as it indicates complications which are described below. The blood pressure is in most cases low. The reflexes are generally increased. The pupils are dilated in bad cases. Patients are mostly quite conscious and convulsions occur very rarely. The skin is mostly pale but in laryngeal lesions it is cyanotic. In the course of time the face turns red when fever appears as a result of complications. In very serious cases death occurs in a few hours with a subnormal temperature. Diarrhoea and bloody faeces are rare and only occurs when there is severe corrosion of the duodenum and jejunum. The urine need not necessarily be alkaline and is sometimes acid in serious fatal cases. An alkaline reaction of the urine often sets in a few hours after poisoning but is transitory. There is protein in the urine in serious cases and often also acetone due to inanition or to decomposition of protein.

The direct causes of death in alkali poisoning may be shock, collapse, glottis oedema, pneumonia, exhaustion, perforation, haemorrhage, mediastinitis, pericarditis and (or) peritonitis without perforation. The maximum number of fatalities fall within the first three days and on the eighth to tenth days according to Balász (1934). The shortest period within which death occurred from alkali poisoning was two hours.

The following complications may follow the swallowing of caustic alkalis:—(1) Stenosis of the oesophagus, pylorus and small intestine. (2) Leschke (1932) states that cancer of the stomach may develop in some cases. (3) Inflammatory and purulent phlegmones of the oesophagus and the stomach which may pass over on to the adjoining organs resulting in pleuritis, pericarditis and peritonitis. (4) Extensive internal haemorrhage due to corrosion of large blood vessels. This is rare in comparison with extensive bleeding in cases of parenchymatous haemorrhage. (5) Abortion is rare. (6) Perforation of the oesophagus, stomach wall and (or) intestinal wall resulting in phlegmones, perioesophagitis, pneumonia, pericarditis, pleuritis and peritonitis. (7) Pronounced glottis oedema necessitating tracheotomy. (8) Toxic effects on the heart due to absorption of decomposition products from necrotic tissues. (9) Shock and collapse. (Fröhner, 1919; Lander, 1926; Lewin, 1929; Van Itallie and Bylsma, 1930; Glaister, 1931; Leschke, 1932 and 1934; Rentz, 1933; Fazekas, 1934 and 1937; Balasz, 1934; Schranz, 1934; Smith and Cook, 1934; Renvis, 1935.)

The following table is compiled from information supplied in Balász's (1934) publication and is of great value in differentiating between acid and alkali poisoning:—

Acids.	*Caustic Alkalis.*
1. Coagulate protein of tissues and partly prevent further corrosion.	1. Dissolve tissue protein and enhances further corrosion.
2. Crust is hard.	2. Lesion is soft, transparent and slimy (moist). If corrosion is severe the area may be reddish-brown or bright-red.
3. Very little or no oedema.	3. Pronounced, jellylike oedema.
4. Lips rarely swollen.	4. Lips almost always swollen.
	5. Infections are more frequent in alkali poisoning as the lesions are more favourable for bacterial growth. Hence perioesophagitis and mediastinitis with perforation are more frequent in alkali than in acid poisoning.

The symptoms of alkali poisoning in animals are similar to those described in human beings.

C. *Post Mortem Appearances.*

These depend upon the concentration and quantity of alkali swallowed and are evident from the description of the symptoms.

D. *Detection of Caustic Soda.*

Reaction of specimen is strongly alkaline. Vomit is the best specimen for analysis, and also stomach contents in cases where no chemical antidotes were administered. The specimen, if fluid, should be concentrated by evaporation and then extracted with absolute alcohol and filtered. The filtrate is then evaporated to dryness and tested for the alkali suspected. The dialysis method could also be used. In protracted cases it is difficult and often impossible to detect the alkali which caused the poisoning.

E. *Treatment of Alkali Poisoning.*

Tracheotomy should be performed in cases of glottis oedema. In less severe cases stomach lavage could be executed with small quantities (200 c.c.) of weak acid solutions (diluted vinegar, acetic acid). Milk and eggs should be administered and Belladonna (atropine) to inhibit gastric and intestinal secretion and motility. It is of importance to treat the weak heart. Morphine injections should be given to allay pain, excitement, incessant vomiting and sleeplessness. Infusions are essential in order to replace the loss of fluid. Serious cases of stenosis of the oesophagus, pylorus and small intestine should be operated upon.

III. Summary.

1. Poisoning with weak and caustic alkalis is reviewed and discussed.

2. Analysis of a specimen of milk maliciously poisoned with caustic soda is described.

3. An account is given of (*a*) experiments in which varying quantities of caustic soda were added to fresh cow's milk and also of experiments conducted upon rabbits with this milk. (*b*) The effects of different concentrations of caustic soda on rabbits.

4. The continuous taking of alkaline stomach powders may have serious consequences.

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V. References.

BALASZ. J. (1934). Laugen-Vergiftungen (Selbstmorde). *Samml. V. Vergiftungsfällen*, Bd. 5, c. 21.

COPE, C. L. (1936). Alkali poisoning. *Brit. Med. Jour. Nov.* 7, pp. 914-917.

DAVIES, G. L. (1939). Alkali poisoning. *Brit. Med. Jour.*, 19 Aug., p. 424.

FAZEKAS, J. (1934). Ätzlaugen-Vergiftung (Doppelter Mordversuch). (*Deut. Zeitschr. gericht. Med.* 23, 194, 1934) *Samml. v. Vergiftungsfällen*, Bd. 5, A. 446.

FAZEKAS, I. Gy. v. (1937). Die Veränderungen des Blutchemismus bei experimenteller Laugenvergiftung. *Arch. Expt. Path. und Pharm.*, 184 Bd., p. 587-604.

FROHNER, E. (1919). Lehrbuch der Toxikologie. Verlag von Ferdinand Enke, Stuttgart.

GLAISTER, J. (1931). A text-book of Medical Jurisprudence and Toxicology. E. and S. Livingstone, Edinburgh.

HELLER, V. G. (1932). Saline and alkaline waters. *Jour. Nutrition*, Vol. 5, pp. 421-429.

HELLER, V. G. (1933). The effect of saline and alkaline waters on domestic animals. *Expt. Sta. Bull. No.* 217, Dec. 1933. Agric. and Mech. College, Oklahama, U.S.A.

HELLER, V. G. and C. H. LARWOOD (1930). Saline drinking water. *Science*, Vol. 71, pp. 223-224.

JOOS, G. und MECKE, W. (1934). Mineralbilanz bei einer Natriumbicarbonat-alkalose. Arch. Expt. Path. u. Pharm., 174 Bd., pp. 687-694.

KOBERT (1906). Lehrbuch der Intoxilnationen. Verlag von Ferdinand Enke, Stuttgart.

LANDER, G. D. (1926). Veterinary Toxicology. Bailliere, Tindall and Cox. London.

LESCHKE, E. (1932). Fortschritte in der Erkenntnis und Behandlung der Wichtigsten Vergiftungen. *Münch. Mech. Wochenshr.*, 79 Jhrg., pp. 266-267.

LESCHKE, E. (1934). Clinical Toxicology. J. and A. Churchill, London.

LINTON, R. G. and A. N. WILSON (1933). Poisoning of pigs by Sodium bicarbonate in flour sweepings. *Vet. Jour.*, Vol. 89, p. 80-82.

LITTLE, L. L. (1937). A simple plant method of estimating the *alkaline* constituents of washing powders and washing solutions containing mixed alkalis. *Jour. Dairy Sci.*, Vol. 20; pp. 83-99.

REID, H. A. (1921). Poisoning of sheep by soda. *Vet. Jour.*, Vol. 77, pp. 177-180.

RENTZ, E. (1933). Wie wirken akute and chronische Säure—bzw. Alakalizufuhr auf den Organismus? *Arch. Expt. Path. u. Pharm.*, Bd. 173, pp. 605-613.

RENVIS, Ed. (1935). Wie wirken akute und chronische Säure—bzw. Alkalizufuhr auf den Organismus? (*Latv. biol. Biedi Raksti.*, 4, 105-110.) *Ber. Ges. Phys. und Expt. Pharm.*, Bd. 89, p. 438.

SCHRANZ, D. (1934). Laugenätzung, eigenartige Fälle. (*Deut. Zeitschr. für gericht. Med.*, Bd. 23, S. 152, 1934). *Samml. v. Vergiftungsfällen*, Bd. 5, A. 445.

SMITH, S. and W. G. H. COOK (1934). Taylor's Principles and Practice of Medical Jurisprudence. J. and A. Churchill, Ltd. London.

VAN ITALLIE, L. en U. G. BYLSMA (1930). Toxicologie en Gerechtelijke Scheikunde. D. B. Centen's Uitgevers Maatschappij, Amsterdam.

WITTER, J. F. (1936). A preliminary report on the injurious effect of sodium bicarbonate in chicks. *Poultry Sci.*, Vol. 15, pp. 256-259.

Section VI.

Nutrition.

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The Biological Value of White Fishmeal as Determined on Growing Sheep and Rats.

By D. B. SMUTS and J. S. C. MARAIS, Section Nutrition, Onderstepoort.

FISHMEAL as a feed for animals has probably first been used in Norway. Since 1905 fishmeal, as distinct from fish manure, was manufactured in appreciable quantities in England. It was, however, not until 1916, that it became a popular ingredient in rations for livestock. It was soon demonstrated that this feed was exceptionally good for young growing animals and poultry. Davidson (1928) showed that the value of fishmeal for feeding of pigs is associated with the protein and particularly with the amounts and proportions of mineral ingredients. By replacing fishmeal with vegetable proteins in the ration of pigs he obtained as good results as with fishmeal, if the former ration is supplemented by a mineral mixture. The same quality carcase as that obtained with fishmeal, could not be established. Plimmer and Rosedale (1934) determined the relative nutritive value of different proteins on chickens by the growth method. The value of these protein feeds was calculated numerically from the equation $1^2/TP$, where 1 equals increase in weight, T length of experiment and P protein consumption. On this basis they classified the proteins as follows:—caseinogen 100, fishmeal 85·3, meatmeal 62·8, wheatgerm 68, bloodmeal 48, lucerne 25·6. From these figures it appears as if fishmeal is a good protein for poultry. However, Halpin and co-workers (1936) found that excessive quantities of fishmeal in the ration of poultry caused "crippled" feet. Carbone (1937) calculated the composition of fishmeal from various sources and concluded that it is not such a satisfactory concentrate for beef cattle and milk cows as oil seed cakes, since it does not give such good growth. It is, however an excellent feed for pigs. Monroe, Krauss and Hayden (1937) compared white fishmeal with linseedmeal as a source of protein in the ration of dairy cattle from 6 months of age until the second calving stage. They found no significant difference in growth and milk production, although the health and general vigour of calves from the fishmeal group were slightly better.

It appears as if the quality of fishmeal is largely determined by the type of product used and the method of manufacturing. Bethke and Wieder (1934) found that cooking as well as high temperature destroy the vitamin content. The protein seems also to be effected. Thus the protein of vacuum dried fishmeal, as determined by these authors was superior to these of flame dried

fishmeals. Oshura and Itaya (1938) observed that the digestibility of fishmeal protein is highest for steamdried and lowest for flame dried and roasted fishmeals. Sundried fishmeal has a much higher NH_3 content than machine dried fishmeal. Schneider (1932) by experiments on rats and pigs found that the protein of vacuum dried white fishmeal has a higher digestibility and is better utilizable than steam dried and flame dried Menhadden fishmeal. It is interesting in this respect that Davies (1936) found by chemical analysis of the crude protein fraction of fishmeals, that these meals are higher in non-protein nitrogen compounds than either meatmeal or blood meal. It is possible that flame drying increases the non-protein nitrogen compounds and hence decreases the digestibility of the protein as was found by the above workers. Swaminathan (1938) found a relationship between the non-protein content of grasses and the digestibility of the protein. A high non-protein content invariably points to a lower digestibility.

Experimental.

White fishmeal which is reported on in this study is a product recently put on the South African market. It contains an average of 67 per cent. protein. The biological utilization of its nitrogen was determined on growing sheep and rats. The weights of the sheep varied from 26 to 31 Kgm. The endogenous nitrogen and metabolic fecal nitrogen were determined previously on these sheep and the results then obtained were utilized in the calculation of the biological value of the protein of fishmeal. With rats the endogenous and metabolic fecal nitrogen were determined in a separate period prior to the actual metabolism period on fishmeal. The composition of the rations is given in Table 1. Wheat straw has now been introduced into our nitrogen low diets for sheep, since we have found no effect on the endogenous nitrogen excretion.

Table 1.

Composition of Rations on Percentage Bases.

	Sheep.	Rats.	Percentage.
White fishmeal	15·0	11·9	—
Wheat straw	50·0	—	—
Dex. starch	31·5	63·1	72·0
Sucrose	—	10·0	10·0
Butter fat	—	8·0	8·0
Codliveroil	0·5	2·0	2·0
Harris Yeast	—	2·0	2·0
Salt mixture*	—	2·0	2·0
Boneash	2·0	—	—
NaCl	1·0	1·0	1·0
Agar	—	—	3·0
Total	100·0	100·0	100·0
Per cent. Nitrogen	2·19	1·50	0·20

* New salt mixture of Hubbel, R., Mendel, J. B. and Wakeman, A. J. (J. Nutr. 14-273-285, 1937).

Discussion of Results.

The metabolism data and the calculation of the biological value, apparent and true digestibilities of the nitrogen of white fishmeal on rats are given in Table 2. The same data pertaining to sheep are given in Table 3. Rats were put for 8 days on a nitrogen low ration, the composition of which is given in Table 1, and then on a collection period of 8 days on the same ration. The endogenous Nitrogen per 100 gm. weight and the metabolic fecal nitrogen per grm. food consumed, determined in this period were utilized in calculating these fractions in the subsequent protein period. After a preliminary period of 11 days on the protein period, collection was carried out over a period of 8 days on the same ration. As will be seen from Table 2 the average apparent and true digestibilities for the 6 rats were 79 and 97 per cent. respectively. The apparent digestibility of vacuum dried white fishmeal on rats as reported by Schneider is 80·7. This value is very nearly the same as ours. Since the apparent digestibility does not take into account the body's contribution of nitrogen in the total fecal excretion of nitrogen, it is not a real measure for the true digestibility of the fishmeal nitrogen. For this reason the true digestibility has been determined, the value of which is 97 per cent. Actually then the white fishmeal nitrogen is 97 per cent. digested. The average biological value for the 6 rats is 90. This figure is somewhat higher than the vacuum dried white fishmeal of Schneider (1932) on which he found an average value of 84. This difference is very small. if it is taken into account that his meal was fed at 10 per cent. and ours at 9 per cent. level.

Referring to the metabolism data on sheep as reproduced in Table 3, it is evident that the digestibilities as well as the biological value are lower than in rats. It must be noted, however, that the level of protein feeding in the case of sheep is approximately 14 per cent., where it is only 9 per cent. in the case of rats. Whether this difference in level alone is the cause of the lower values is impossible to say. It is quite possible as Timarin suggested, that the difference may be due to a different intensity of enzyme action or to a varying degree of wastage of digestive protein by putrefactive fermentation according to the rates of passage of the food through the alimentary canal of these two species of animals. The alimentary canal of the pig is probably less complicated than the sheep, and resembles more closely that of the rat. Schneider (1932) at a 12 per cent. level of white fishmeal obtained an apparent digestibility of 80 per cent. for pigs in comparison with our value of 63 for sheep. The rat in his experiments and ours showed values of 84 and 79 per cent. digestibility respectively. The true digestibility of the nitrogen contained in white fishmeal in our experiment with sheep is 87 per cent.

As will be seen from Table 3, the average biological value of white fishmeal with sheep is 74. This is lower than the value of 83 reported by Schneider (1932) on pigs. It would appear therefore as if data obtained from rats can be applied to the pig, but that the application of such data is doubtful with animals like the sheep, having a more complicated alimentary tract.

TABLE 2.

Biological Value of an 8 per cent. White Fishmeal Ration.
Nitrogen Metabolism Data and the Calculation of the Biological Value.

Animal No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Focal N.	Body N in Feces. Per gm. Food.	Body N in Feces. Per Day.	Food N in Feces.	Absorbed Nitrogen.	Daily Urinary N.	Body N in Urine. Per 100 gm.	Body N in Urine. Per Day.	Food N in Urine.	Food N Retained.	Biological Value.	Apparent Digestibility.	True Digestibility.
	gm.	gm.	gm.	gm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.			
N low ration containing 0·21 per cent. N.																		
1	142	130	136	7·8	—	19·1	2·45	—	—	—	3·5	23·2	—	—	—	—	—	—
2	127	120	124	6·9	—	22·0	3·19	—	—	—	31·4	25·3	—	—	—	—	—	—
3	87	80	84	5·4	—	13·8	2·56	—	—	—	18·7	22·3	—	—	—	—	—	—
4	98	89	94	5·5	—	15·7	2·85	—	—	—	23·4	24·9	—	—	—	—	—	—
5	92	88	90	6·1	—	17·3	2·84	—	—	—	22·5	25·0	—	—	—	—	—	—
6	81	79	80	6·4	—	14·7	2·30	—	—	—	19·6	24·5	—	—	—	—	—	—
White Fishmeal Ration containing 150 per cent N.																		
1	160	184	172	12·3	184·5	37·8	2·45	30·1	7·7	176·8	52·0	23·2	39·9	12·1	164·7	93	80	96
2	137	167	152	12·3	184·5	43·8	3·19	39·2	4·6	179·9	56·4	25·3	38·5	17·9	162·0	90	76	98
3	95	117	106	9·1	136·5	26·4	2·56	23·3	3·0	133·5	36·6	22·3	23·6	13·0	120·5	90	81	98
4	94	123	109	9·3	139·5	29·8	2·85	26·5	3·3	136·2	40·3	24·9	27·1	13·2	123·0	90	79	98
5	80	118	99	10·6	159·0	31·5	2·84	30·1	1·4	157·6	44·3	25·0	24·8	19·5	138·1	88	80	99
6	91	124	108	11·5	172·5	35·4	2·30	26·5	8·9	163·6	45·8	24·5	26·5	19·3	144·3	88	80	95
															AVERAGE..........	90	79	97

The biological value of 74 nevertheless justifies the conclusion that the protein of white fishmeal is well constituted, and utilized exceedingly well by young sheep.

Summary and Conclusions.

By means of nitrogen metabolism experiments with white fishmeal on rats and sheep, it was found, that the apparent and true digestibilities of the white fishmeal protein are respectively 79 and 97 per cent. with rats, and 63 and 87 per cent. with sheep.

The biological value as determined by rats at approximately 9 per cent. protein level is 90 and for sheep at approximately 14 per cent. level 74.

It is concluded that white fishmeal is a good protein feed for growing sheep.

REFERENCES.

BETHKE, R. M. and WILDER, O. H. (1934). Effect of the method of manufacture on the nutritive value of fishmeals as determined by growth studies on chickens. *J. Agric. Res.* (U.S.), Vol. 49, No. 8, pp. 715-722.

CARBONE, E. (1937). La forina di pesce nele alimentazoine del bestiana. *Rio. Zootec.*, Vol. 14, pp. 429-435.

DAVIES, W. L. (1936). The crude protein fraction of fishmeals and other meatmeals. *The Analyst*, Vol. 61, No. 725, pp. 512-575.

DAVIDSON, H. R. (1928). Substitutes for fishmeal in the rations of fattening pigs. *J. Ministry Agric.*, Vol. 53, p. 5.

HALPIN, J. G., HOLMES, C. E., ELVEHYEM, C. and HART, E. B. (1936). Too much fishmeal caused crippled feet in chickens. *Wisc. Agric. Exp. St. Bull.* 430.

MONROE, E. F., KRAUSS, W. E. and HAYDEN, C. (1937). White fishmeal for growth and milk production. *Bi-monthly Bull. Ohio Agric. Exp. St.* No. 22, pp. 46-52.

PLIMMER, H. A. and ROSEDALE, J. L, RAYMOND, W. H., LOWNDES, J. (1934). The relative values of proteins. *Biochem. J.*, Vol. 28, No. 5, pp. 1863-1886.

OSHUMA, K. and ITAYA, S. (1938). The relation of qualities and the manufacturing method of fishmeal and scrap. *J. Agric. Chem. Soc. Japan.* 1938, Vol. 14, pp. 512-517.

SCHNEIDER, B. H. (1932). Nitrogen balance studies with various fishmeals. *J. Agric. Res.* (U.S.), Vol. 44, No. 9, pp. 723-732.

SWAMINATHAN, M. (1938). The relative amounts of the protein and non-protein nitrogenous constituents occurring in foodstuffs and their significance in the alteration of the digestibility coefficient of protein. *Indian J. Med. Res.*, Vol. 25, pp. 847-855.

TIMARIN, S. (1938). Versuche über die Verwertung von Eiweiss durch Wächsende Kinder. *Landwirtsch. Versuch. Stal.*, No. 129, pp. 124-150.

TABLE 3.

Metabolism Data of young Sheep on Fishmeal Ration Containing 2·19 per cent Nitrogen.

Animal No.	Average Weight.	Food Consumption.	Dry Matter Intake.	Nitrogen Intake.	N. in Feces.	Metabolic Fecal N. Daily.	Absorbed N.	N. in Urine.	Endogenous N. Daily.	Food N. Retained.	Biological Value.	N. Balance.	Apparent Digestibility.	True Digestibility.
	Kgm.	Gm.	Grm.	Grm.	Grm.	Grm.	Grm.	Grm.	Grm.	Grm.				
30	24	490	447	10·73	3·66*	2·50	9·57	3·56	1·01	7·02	73	3·51	66	89
31	31	490	447	10·73	4·15	2·68	9·26	3·55	1·40	7·11	77	3·03	61	86
32	26	490	447	10·73	4·06	2·59	9·28	4·00	1·35	6·63	71	2·70	62	86
33	31	490	447	10·73	3·95	2·68	9·46	4·15	1·61	6·92	73	3·33	63	88
34	27	490	447	10·73	3·96	2·68	9·45	3·45	1·24	7·24	77	3·32	63	88
											74	—	63	87

Section VII.

Pathology.

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Quantitative Studies upon Porphyrin Excretion in Bovine Congenital Porphyrinuria (Pink Tooth) No. 2.

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THE first known living cases of bovine congenital porphyrinuria were described by Fourie (1936) from the clinical and pathological, and by Rimington (1936), Rimington and Fourie (1937), Rimington and Roets (1937) from the bio-chemical point of view. Rimington, Roets and Fourie (1938) presented certain details concerning the quantitative excretion of porphyrins in these cases, but at that time the authors felt that the data were incomplete and the results reported in this paper are intended to be supplementary to those previously presented.

Fischer, quoted by Schumm (1919), found ·17 gm. to ·5 gm. of porphyrin pigments daily in the urine of Petry when he made the determinations during different months of the year (November to February). Schumm (1919) examined the urine of Petry during the summer and during the winter and found more or less the same amounts (circa 0·4 gm.) on both occasions.

Beyond this very little seems to be known concerning the quantitative excretoin of porphyrins in congenital porphyrinuria in man and animals. Recently, however, a good deal of work is reported concerning the quantitative determination of porphyrins in the case of normal humans as well as in cases of man and animals suffering from various diseases, e.g. Dobriner (1937), Brugsch (1938), Rimington (1938), Grotepass (1938), Mertens (1937), and Roets (1938). In these papers notable advances are recorded concerning the methods employed in order to determine quantitatively the porphyrins excreted.

In this paper the authors intend to record results concerning the quantitative porphyrin excretion during different times of the year by bovines suffering from congenital porphyrinuria (Pink

Tooth), and at the same time data are presented concerning the relative porphyrin excretion in bovine congenital porphyrinuria in a completely normal bovine and in apparently* normal bovines.

Details of Experiments.

The animals were maintained in metabolism stables on a daily ration of 5 pounds veld hay in the morning and 8 pounds crushed maize with 2 pounds of green food in the afternoon. The animals were watered twice daily and the amount of water consumed was carefully measured.

The amount of faeces passed and the amount of urine excreted were carefully weighed and measured every 24 hours. The animals were usually under observation for a period of 14 days, but determinations were not recorded for the first 24-48 hours of the experiment, as the animals were sometimes a little bit upset when they were confined to the metabolism stables, resulting in some cases in diarrhoea. During this 24-48 hour period they were therefore allowed to settle down to the conditions of watering, feeding, etc.

Daily quantitative porphyrin determinations of faeces and urine were made in the case of the known pink tooth animals 7016, 7017 (see Fourie, 1936) in the same way as this was done by Rimington, Roets and Fourie (1938).

On account of the low level of porphyrin excretion in the normal animal 6384 and in the apparently normal animals 7022, 7393 and 7356 a slightly modified method of dealing with the faeces and urine was made use of. Before giving details of the method in which the faeces and urine were actually handled, it is perhaps just as well to give a brief history of these animals.

No. 6384 is a red heifer entirely unrelated to the porphyrin animals, but the bull 7015 (see Fourie, 1936) which is known to transmit the condition is being mated to her, in an attempt to produce further porphyrin carriers for the study of the genetics of congenital porphyrinuria.

No. 7393 is a carrier, being the daughter of a porphyrin cow.

No. 7022 is a daughter of Bull 7015 and thus possibly a carrier—she has, however already produced 2 calves from matings to her own father, but both calves are clinically normal.

No. 7356 is a heifer and one of the two calves of 7022.

The last three animals (7393, 7022, and 7356) are therefore listed as apparently normal as one certainly and two possibly may be carriers of the recessive character.

* Apparently normal is here used for animals which in some cases are actually known to be carriers of the anomaly, and in other cases for animals which may be carriers, since they are daughters of the bull 7015 (see Fourie, 1937) which is known to be a carrier of the recessive character. A paper concerning the hereditary nature of the condition as a recessive character will be published by one of us (Fourie) at a later date.

In addition to these three animals in this group, a further animal was added, viz. No. 7597. He is a bull which is showing very marked photosensitization on exposure to the sun. His teeth are only slightly discoloured if at all and porphyrin excretion in the faeces and urine is on a low level. This animal, however, has a remote common ancestry to bull 7015 and is at present being regarded as a very suspicious but atypical case of congenital bovine porphyrinuria. Confirmation of this is being awaited, pending the results of certain breeding experiments already commenced.

Method of Dealing with Urine and Faeces of Normal and Apparently Normal Animals, etc.

Urine.

A representative sample of 500 c.c. of urine from each animal was *collected* at the end of each 24 hour period. It was stored in the ice chest and every 24 hours an aliquot of 500 c.c. of that day was added to that collected on previous days, until at the end of four days 2,000 c.c. of urine was available for examination from each animal. Acetic acid was then added up to a concentration of 5 per cent., and the solution extracted in a big Kutcher-Steudal continuous extraction apparatus for 24 hours. The ethereal solution was thoroughly washed in a separatory funnel with water containing potassium acetate, in order to remove the acetic acid and any soluble urinary pigments. The water washings were, however, rewashed with fresh ether to safeguard against any possible porphyrin loss. Any porphyrin so obtained in ether solution was then added to the main ether solution containing porphyrin so that the final ether solution usually had a volume of approximately 750 c.c. From this solution the porphyrins were transferred to acid solution by repeatedly adding to the former 10 to 15 c.c. of 5 per cent. HCl and shaking thoroughly. Negative fluorescence in ultra violet light indicates when the end point has been reached. The entire acid solution about 50 c.c. in volume, was transferred to a 100 c.c. separatory funnel, potassium acetate added as before, the porphyrins again shaken into ethereal solution and the water washings referred to above repeated The porphyrins from this final solution, which was approximately 30 c.c. in volume was shaken into 5 to 10 c.c. of 5 per cent. HCl, and the intensity of the 550 band compared spectroscopically with a porphyrin standard of known value in the usual way.

Because of the small amount of porphyrin present in these animals, two of them (one 7597 the suspected porphyrin sufferer and the other 6384, completely normal) were used for further investigation concerning the presence of uroporphyrin, by adding acetic acid to their ether soluble porphyrin extracted urine to a concentration of 5 per cent. and filtering through alumina absorption columns, but no uroporphyrin could be detected by eluting with dilute alkali.*

* This method was devised and employed by one of us (Roets) in June (1938). Since this article was written up in November (1938) Dobriner and Rhoads (1938) independently used practically the same method to determine urinary coproporphyrin in the urine of man.

Faeces.

50 gm. of a representative sample of faeces from each animal was collected daily in acetic acid. This was kept in the dark and after 4 days the combined 200 gm. from each animal was extracted in the usual way with acetic acid and ether. The porphyrin was transferred to 5-20 c.c. of 5 per cent. HCl (depending on the porphyrin content). This solution was shaken with chloroform to remove soluble pigments and the copro-porphyrins determined.

The handling and sampling of the faeces and urine were otherwise carried out as described by Rimington, Roets and Fourie (1938).

Results Obtained.

Faeces and urine from the two pink tooth animals 7017 and 7018 were examined from 9/11/37 to 19/11/37 and again from 14/2/38 to 24/2/38. That from the third pink tooth animal 7016 was examined from 8/12/37 to 23/12/37. The results are recorded in tables 1, 2 and 3.

In Figs. I, II and III the daily total porphyrin, daily total copro-porphyrin and the daily total uroporphyrin, with corresponding averages for the three animals 7017, 7018 and 7016 are graphically presented.

The I and III series isomers of coproporphyrin were separated and quantitatively determined in urine samples of 7017, 7018, and in faeces samples of 7017, 7018, 7022 (apparently normal, possibly a carrier) and 7597 (suspected pink tooth bull) (Table 4) using the method described by Rimington, Roets and Fourie (1938). A portion of urine and of faeces excreted during a 24-hour period was taken for this purpose.

Analysing the figures presented in Tables 1, 2 and 3 there does not appear to be a consistent relationship between the daily porphyrin excretion and total daily faeces or urine output.

In the case of animal 7017, in which the greatest porphyrin excretion is recorded there was, after an initial rise in porphyrin excretion, a steady decline for the first few days after the animal was placed in the metabolism stable (admittedly under completely different environmental conditions than the animals were used to). Thereafter and until the completion of the ten day period the porphyrin excretion steadily increased, this being mainly due to an increase in the copro-porphyrin, as the uroporphyrin fluctuated irregularly during that time. This examination occurred during the early summer.

When the animal was again examined for ten days during the late summer, there was again an initial porphyrin decrease, but this time more regularly progressive than the previous time, in spite of a somewhat marked increase in the uroporphyrin at times. Subsequently, however, the porphyrin fluctuation was rather irregular.

In the other two animals (7018 and 7016) the porphyrin excretion fluctuated widely and irregularly from day to day.

TABLE 1.

(Bovine 7017.)

Date.	FAECES.			URINE.							
	Weight in gm.	Coproporphyrin.		Volume in c.c.	Coproporphyrin.		Uroporphyrin.		Total Coproporphyrin in gm.	Total Uro- + Coproporphyrin in gm.	Water Intake in Litres.
		Mg. per 100 gm.	Total in gm.		Mg. Per 100 cc.	Total in gm.	Mg. per 100 c.c.	Total in gm.			
9–10/11/37.......	7,410	8·0	0·5928	2,860	3·25	0·093	2·667	0·0763	0·6858	0·7621	11·5
10–11/11/37.......	8,750	9·6	0·8400	3,580	1·925	0·0689	1·867	0·0668	0·9089	0·9757	16·5
11–12/11/37.......	6,760	7·2	0·4867	4,825	·733	0·0354	1·333	0·0643	0·5221	0·5864	19·5
12–13/11/37.......	7,260	5·688	0·4129	2,190	2·167	0·0475	2·133	0·0467	0·4604	0·5071	14·5
13–14/11/37.......	6,810	8·125	0·5533	1,970	4·875	0·0960	2·667	0·0525	0·6493	0·7018	16·5
14–15/11/37.......	7,010	8·75	0·6134	3,705	4·5	0·1667	2·4	0·0889	0·7798	0·8687	17·75
15–16/11/37.......	6,090	12·0	0·7308	5,190	1·333	0·0692	1·667	0·0865	0·8000	0·8865	16·0
16–17/11/37.......	7,360	11·5	0·8464	4,150	2·75	0·1141	1·8	0·0775	0·9605	1·0380	20·0
17–18/11/37.......	6,310	14·25	0·8972	5,960	1·75	0·1043	2·0	0·1192	1·0035	1·1247	20·0
18–19/11/37.......	6,440	15·0	0·9660	4,560	2·333	0·1064	2·233	0·1034	1·0724	1·1758	2·75
AVERAGE....	7,020	10·011	0·6944	3,899	2·562	0·0915	2·077	0·0782	0·7843	0·8627	15·5
14–15/ 2/38.......	5,440	30·0	1·6320	1,445	5·5	0·0795	9·0	0·1301	1·7115	1·8416	16
15–16/ 2/38.......	6,820	20·0	1·3640	6,560	2·2	0·1443	3·75	0·2460	1·5083	1·7543	16·8
16–17/ 2/38.......	8,070	17·5	1·4123	2,310	6·0	0·1386	5·25	0·1213	1·5509	1·6722	6·0
17–18/ 2/38.......	6,670	18·0	1·2006	4,400	2·8	0·1232	4·5	0·1980	1·3238	1·5218	14·6
18–19/ 2/38.......	7,520	14·0	1·0528	4,830	2·8	0·1352	3·2	0·1546	1·1880	1·3426	4·4
19–20/ 2/38.......	8,980	16·0	1·4368	3,290	5·4	0·1777	4·5	0·1481	1·6145	1·7626	14·4
20–21/ 2/38.......	11,520	14·0	1·6128	2,680	5·25	0·1407	4·5	0·1206	1·7535	1·8741	14·4
21–22/ 2/38.......	9,070	12·0	1·0884	—	—	—	—	—	—	—	12·2
22–23/ 2/38.......	8,470	13·75	1·1646	2,600	4·5	0·1170	4·0	0·1040	1·2816	1·3856	8·0
23–24/ 2/38.......	9,220	16·25	1·4983	2,570	5·5	0·1414	4·2	0·1079	1·6397	1·7476	9·0
AVERAGE....	8,178	17·15	1·3463	3,409	4·217	0·1331	4·77	0·1478	1·5080	1·6558	11·58

TABLE 2.

(Bovine 7018.)

Date.	FAECES.			URINE.					Total Copropor-phyrin in gm.	Total Uro- + Copropor-phyrin in gm.	Water Intake in Litres.
	Weight in gm.	COPROPORPHYRIN.		Volume in c.c.	COPROPORPHYRIN.		UROPORPHYRIN.				
		Mg. per 100 gm.	Total in gm.		Mg. Per 100 cc.	Total in gm.	Mg. per 100 c.c.	Total in gm.			
9–10/11/37.......	8,810	3·57	0·3179	2,640	0·714	0·0189	1·143	0·0328	0·3368	0·3696	15·75
10–11/11/37.......	10,310	3·8	0·3918	5,270	0·5	0·0264	0·914	0·0482	0·4182	0·4664	17·0
11–12/11/37.......	8,710	1·95	0·1698	4·730	0·429	0·0203	1·143	0·0541	0·1901	0·2442	15·0
12–13/11/37.......	8,610	1·552	0·1336	4,000	0·476	0·0190	1·142	0·0457	0·1526	0·1983	17·75
13–14/11/37.......	8,510	1·8	0·1532	3,235	0·429	0·0130	1·286	0·0416	0·1662	0·2078	21·75
14–15/11/37.......	7,890	3·2	0·2525	3,900	0·771	0·0301	1·143	0·0474	0·2826	0·3300	20·75
15–16/11/37.......	5,740	3·0	0·1722	5,400	1·071	0·0536	1·143	0·0617	0·2258	0·2875	16·25
16–17/11/37.......	6,310	4·063	0·2564	7,160	0·686	0·0491	1·143	0·0818	0·3055	0·3873	19·75
17–18/11/37.......	5,210	3·125	0·1943	9,110	0·714	0·0650	0·914	0·0833	0·2593	0·3426	18·5
18–19/11/37.......	6,360	8·5	0·5406	8,930	0·643	0·0574	0·914	0·0816	0·5980	0·6796	9·0
AVERAGE....	7,646	3·446	0·2582	5,438	0·643	0·0353	1·089	0·0578	0·2935	0·3513	17·15
14–15/ 2/38.......	5,380	15·0	0·8070	8,440	0·594	0·0501	0·686	0·0579	0·8571	0·9150	29·2
15–16/ 2/38.......	7,850	10·0	0·7850	10,040	0·557	0·0559	0·937	0·0941	0·8409	0·9350	15·8
16–17/ 2/38.......	6,300	11·0	0·6930	7,130	0·857	0·0611	0·914	0·0652	0·7541	0·8193	14·4
17–18/ 2/38.......	5,790	12·0	0·6948	16,960	0·245	0·0416	0·353	0·0599	0·7364	0·7963	25·0
18–19/ 2/38.......	8,775	9·5	0·8336	14,380	0·274	0·0394	0·686	0·0986	0·8730	0·9716	14·4
19–20/ 2/38.......	7,970	8·0	0·6576	8,300	0·68	0·0564	0·914	0·0759	0·7140	0·7899	26·0
20–21/ 2/38.......	9,980	8·0	0·7984	7,920	1·114	0·0771	1·143	0·0791	0·8755	0·9546	22·0
21–22/ 2/38.......	8,100	9·5	0·7695	6,580	0·857	0·0564	1·143	0·0752	0·8259	0·9011	16·8
22–23/ 2/38.......	9,670	7·5	0·7253	6,660	0·857	0·0571	1·32	0·0879	0·7824	0·8703	18·0
23–24/ 2/38.......	9,200	7·0	0·6440	7,310	0·743	0·0543	0·875	0·0620	0·6983	0·7603	17·0
AVERAGE....	7,902	9·75	0·7408	9,272	0·678	0·0549	0·897	0·0756	0·7957	0·8713	19·9

TABLE 3.

(Bovine 7016.)

Date.	FAECES.			URINE.					Total Coproporphyrin in gm.	Total Uro- + Coproporphyrin in gm.	Water Intake in Litres.
	Weight in gm.	COPROPORPHYRIN.		Volume in c.c.	COPROPORPHYRIN.		UROPORPHYRIN.				
		Mg. per 100 gm.	Total in gm.		Mg. Per 100 cc.	Total in gm.	Mg. per 100 c.c.	Total in gm.			
8– 9/12/37.......	7,860	1·563	0·1229	2,500	0·767	0·0192	1·467	0·0367	0·1421	0·1788	—
9–10/12/37.......	6,910	3·8	0·2626	3,600	0·686	0·0247	1·143	0·0411	0·2873	0·3284	14·25
10–11/12/37.......	6,800	3·75	0·2550	4,620	0·557	0·0257	1·093	0·0505	0·2807	0·3312	17·75
11–12/12/37.......	7,460	3·6	0·2686	4,320	0·893	0·0386	1·143	0·0494	0·3072	0·3566	16·0
12–13/12/37.......	6,740	2·6	0·1752	5,880	0·513	0·0302	0·8	0·0470	0·2054	0·2524	9·9
13–14/12/37.......	7,410	3·0	0·2223	3,340	0·514	0·0172	1·029	0·0344	0·2395	0·2739	20·0
14–15/12/37.......	10,160	2·7	0·2743	4,400	0·32	0·0141	0·971	0·0427	0·2884	0·3311	16·25
16–17/12/37.......	6,260	3·3	0·2066	3,180	0·571	0·0182	1·6	0·0509	0·2248	0·2757	—
17–18/12/37.......	6,260	3·2	0·2000	4,960	0·374	0·0186	1·016	0·0504	0·2186	0·2690	24
18–19/12/37.......	8,610	3·4	0·2927	6,380	0·314	0·0200	—	—	0·3127	—	21·2
19–20/12/37.......	11,260	2·325	0·2618	4,035	0·42	0·0169	0·914	0·0369	0·2787	0·3156	19·5
20–21/12/37.......	6,940	3·14	0·2186	5,200	0·334	0·0174	0·824	0·0428	0·2360	0·2788	12·0
21–22/12/37.......	8,940	1·95	0·1743	4,000	0·33	0·0132	0·914	0·0366	0·1875	0·2241	20·5
22–23/12/37.......	9,360	1·06	0·0992	3,660	0·238	0·0087	0·857	0·0314	0·1079	0·1393	18·2
AVERAGE....	7,926	2·813	0·2167	4,191	0·488	0·0202	1·059	0·0424	0·2655	0·2888	16·12

FIG. I.—Bovine 7017.

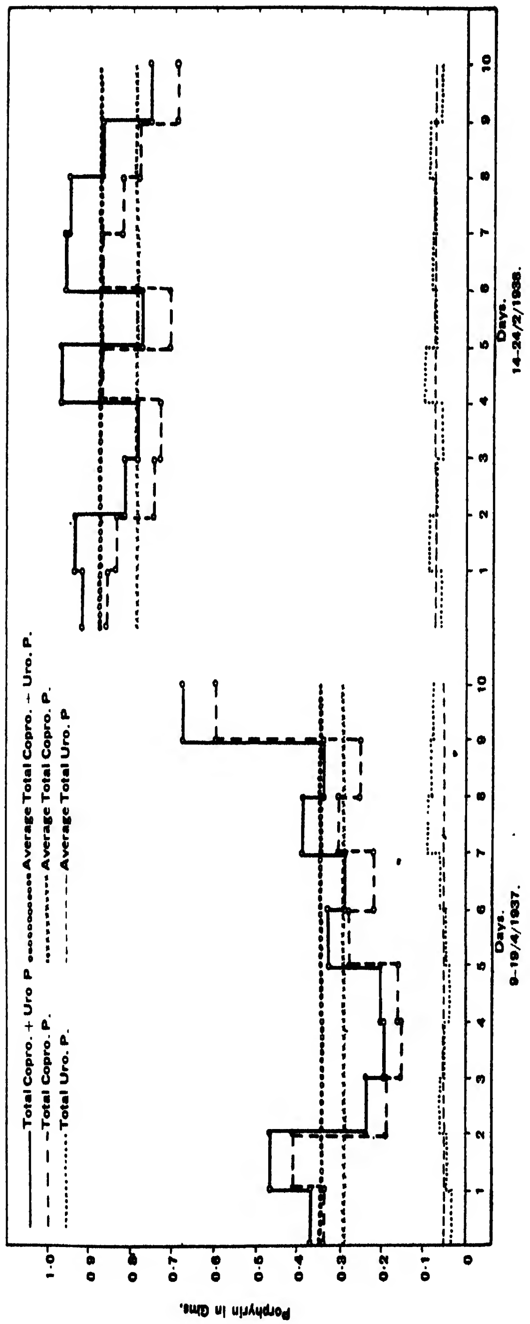

FIG. III.—Bovine 7016.

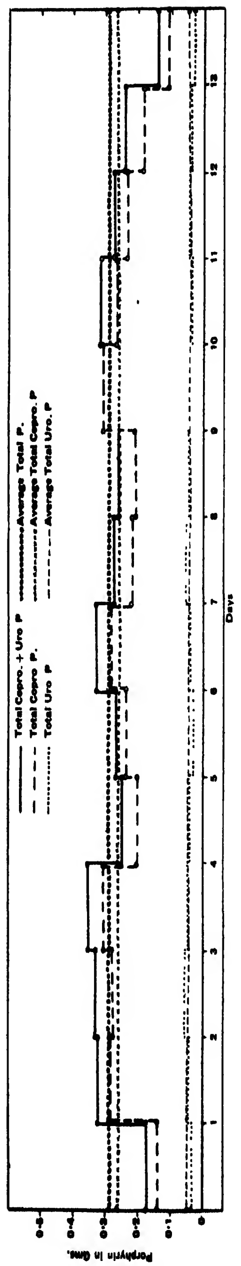

FIG. 11.– Bovine 7018.

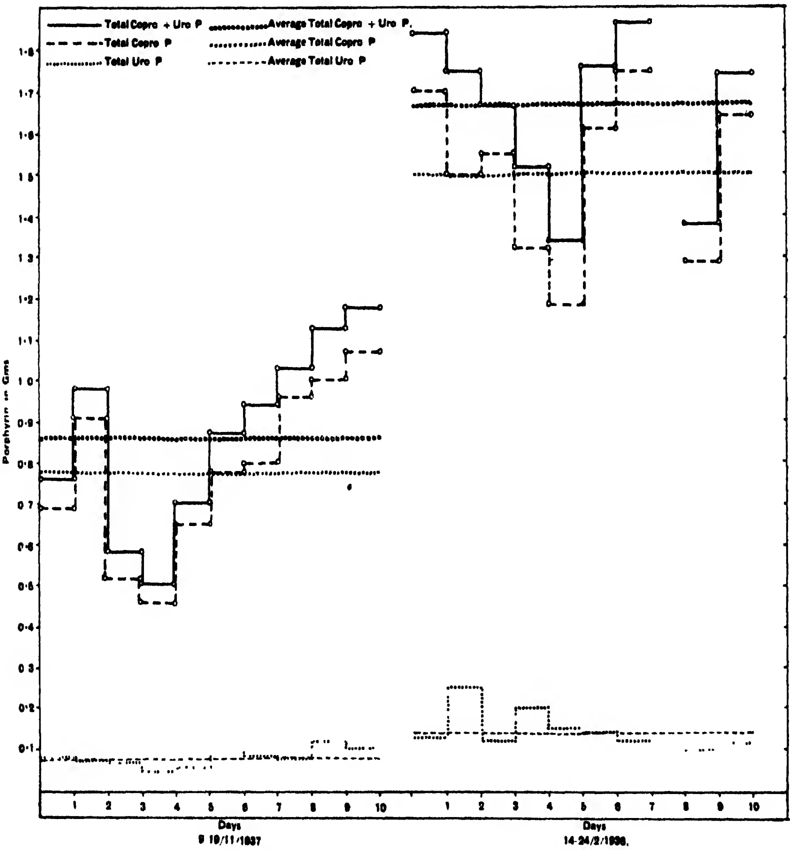

TABLE 4.

Ratio Copro I: Copro III.

No. of Animal.	Date.	Material.	Total Copro. in mg.	Copro. I in mg.	Copro. III in mg.	Ratio of Copro. I : III.
7017	18–19/11/37.	Urine........	52·5	45·0	2·25	1 : 0·05
7018	18–19/11/37.	Urine........	18·75	15·0	2·52	1 : 0·167
7017	23–24/ 2/38.	Faeces.......	41·0	37·5	1·68	1 : 0·04
7018	23–24/ 2/38.	Faeces.......	22·5	20·0	0·765	1 : 0·038
7022	24–25/ 6/38.	Faeces.......	—	0·105	0·065	1 : 0·619
7597	24–25/ 6/38.	Faeces.......	—	0·238	0·30	1 : 1·261

It is perhaps worthy of note that both the animals 7017 and 7018, which were always under observation at the same time, excreted porphyrin at a much higher level when examined during the late summer than was the case during the early summer. It is not considered likely that photosensitization may have been the cause of this, as the animals were only exposed for a very short time to the sun, when they were driven from one stable to the other. That the change in the season may have been a factor, cannot be definitely stated. However, it seems probable that seasonal variation was responsible for the increased porphyrin excretion during the late summer, since a change in food was certainly not a factor, as the two animals under conditions of stabling were given the same food on both occasions.

This higher level of porphyrin excretion in animal 7017 during the late summer was not so much due to a greater urine volume as to a greater porphyrin concentration, whereas in the case of 7018, the concentration of porphyrin in the urine was more or less the same during early spring, but as the total volume of urine was markedly increased (average daily output early spring = 5,438 c.c., average daily output late summer = 9,272 c.c.) the higher uro-porphyrin excretion was definitely due to this greater total urine output. However, in the case of the faeces of both animals the concentration was definitely higher during late summer than during the early spring, whilst the faeces weight remained at more or less the same level on both occasions. In order to eliminate the possibility that an experimental error may have been responsible for the higher values obtained during the late summer, new standards were made up and determinations were made in duplicate, when it was found that the results corresponded in both instances. Whether this seasonal variation in the porphyrin excretion can satisfactorily explain the uneven distribution of the porphyrins in bones of affected animals as described by Fourie (1936), is a point which cannot at the moment be definitely settled from the available information.

In working out the ratio of total to copro-to uro-porphyrin excreted from the daily averages (see Table 5) during the periods of 10 to 14 days the figures obtained are strikingly constant viz. approximately 10:9:1 for the three animals 7016, 7017 and 7018. There is however a deviation from the constant in the case of animal 7018, during the period 9/11/38 to 19/11/38, when the ratio is approximately 10:8:2. This deviation is due to the exceptionally large volume of urine which was excreted by this animal at that time as compared with the volume of urine excreted by this animal subsequently, and as compared with the amounts excreted by the other two animals.

TABLE 5.

No. of Animal.	Date.	Daily Average of Total Porphyrin in gm.	Daily Average Total Coproporphyrin in gm.	Daily Average Total Uroporphyrin in gm.	Ratio of— Total.	Copro.	Uro.
7017	14-24/ 2/38......	1·6558	1·5080	0·1478	10	9·1	0·9
7017	9-19/11/37......	0·8627	0·7841	0·0782	10	9·1	0·9
7018	14-24/ 2/38......	0·8713	0·7957	0·0756	10	9·1	0·8
7018	9-19/11/38......	0·3513	0·2935	0·0578	10	8·4	1·6
7016	8-23/12/37......	0·2888	0·2655	0·0424	10	9·2	1·46

The average coproporphyrin concentration in the urine of the normal animal 6384, which is not in any way related to the congenital porphyrinuric animals, over a period of 8 days is ·056 mg. per 2,000 c.c. of urine. This is a little higher than the concentration in normal human urine as determined by Grotepass (1938), who found the concentration to be ·02 mg. per 1,000 c.c. in 10,000 litres of urine. In man the total average daily coproporphyrin excretion over 9-day periods is 306 to 376 micro-grams (Dobriner, 1937).

The figures obtained for the normal animal 6384 over an 8-day period are: (*a*) total daily average coproporphyrin excreted in the urine—·056 mg. per 2,000 c.c. and (*b*) the total daily average coproporphyrin excreted in the faeces—·056 mg. per 100 mg. When the excreted porphyrin of the apparently normal animals Nos. 7022 and 7393 (both being related to congenital porphyrinuria animals) is compared with that of the normal animal 6384 (see Table 6) it will be seen that the concentrations of coproporphyrin both in urine and faeces are slightly higher viz.:—

7022—urine, ·066 mg. per 2,000 c.c.
faeces, ·11 mg. per 100 gm.

and 7393—urine, ·069 mg. per 2,000 c.c.
faeces, ·105 mg. per 100 gm.

TABLE 6.

Showing concentration of coproporphyrin in faeces and urine of one normal animal (6384), *of three apparently normal animals* (7022, 7356, 7393) *and of one suspected case of Pink Tooth* (7597).

No. of Animal.	Date.	Faeces.		Urine.		
		Weight in gm.	Coproporphyrin mg. per 100 gm.	Volume in c.c.	Coproporphyrin mg. in 2,000 c.c.	Water Intake in Litres.
7022...........	16–20/6/38	40,880	0·102	7,790	0·058	35·8
	20–25/6/38	33,200	0·116	7,790	0·072	37·6
Daily Average...	16–25/6/38	8,231	0·110	1,731	0·066	8·2
7597...........	16–20/6/38	26,140	0·144	11,575	0·147	33·4
	20–25/6/38	41,420	0·15	16,770	0·08	47·2
Daily Average...	16–25/6/38	7,537	0·147	3,149	0·11	8·96
6384...........	11–15/7/38	26,430	0·053	5,330	0·06	21·6
	15–19/7/38	19,050	0·058	13,615	0·051	29·4
Daily Average...	11–19/7/38	5,685	0·056	2,368	0·056	6·4
7393...........	11–15/7/38	28,230	0·111	7,322	0·053	24·6
	15–19/7/38	26,300	0·098	11,500	0·083	43·0
Daily Average...	11–19/7/38	6,059	0·105	2,353	0·068	8·5
7356...........	13–19/7/38	18,640	0·058	10,620	0·073	40·0
Daily Average...	-	3,107	0·058	1,770	0·073	6·7

Whether these values also fall within the limits of normality is not known at the moment.

Animals 7022, 7393, 7356 and 6384 were originally included in these experiments as a group of normal controls but when the porphyrin values of three of them were found to be higher than the completely normal animal 6384 which is not in any way related to porphyrinuric animals, we began to doubt if their porphyrin values can be regarded as normal. This is most unfortunate as we now have values for only one normal animal (6384). However, we hope soon to have results of at least 8 normal animals, which will be examined at different times of the year and it is only when these figures are available that we shall be in a position to state what the quantitative porphyrin excretion in the normal bovine is.

Animal 7356 (known carrier) was also examined but as this animal had rather a profuse diarrhoea at the time of the examination, it is not used for purposes of comparison, as the digestive disturbance may possibly influence the values obtained.

In the case of the suspected porphyrinuric bull 7597 showing marked and continuous photosensitization the porphyrin excretion in faeces and urine is at a higher level than in the normal animal 6384 but very much less than in the undoubted pink tooth cases.

The ratio of coproporphyrin I to coproporphyrin III (see Table 4) in faeces and urine varies from animal to animal. In man Grotepass (1938) puts the average ratio of the I to the III series in normal human urine as 96:87.

Calculating the coproporphyrin III concentration in the urines of 7017 and 7018 excreted during the 18th to the 19th November, 1937, when the examination was made it amounts to 0·111 mg. per 100 c.c. and 0·092 mg. per 100 c.c respectively. This is much higher than the daily average total coproporphyrin concentration of 0·003 mg. per 100 c.c. in the urine of the normal animal 6384 (see Table 6). This suggests that the affected animals excrete increased amounts of both copro I and copro III, but unfortunately we have not been able to establish this definitely as up to now it was not possible to determine the presence of these pigments in faeces and urine of a sufficient number of normal bovines when the same animals are examined at different periods during the year.

Summary.

1. In addition to the daily fluctuation in the amount of porphyrin excreted, porphyrin was found to be excreted at a higher level in two affected animals during the late summer than during the early spring. This was found to be due to a greater porphyrin concentration in the faeces and urine than to an increased amount of faeces or urine.

2. When quantitative porphyrin determinations were made over a period of 10-14 days and the daily averages calculated, it was found that the ratio of uro- plus coproporphyrin to coproporphyrin to uroporphyrin was approximately constant except in one case, where during one period abnormally large amounts of urine were excreted.

3. The ratio of coproporphyrin I to coproporphyrin III varies from animal to animal in both the faeces and the urine of two affected animals (7017 and 7018), one suspicious case of the disease (7597) and one apparently normal animal (7022) which is however related to congenital porphyrinuria cases.

Literature.

BRUGSCH, J. T. (1938). *Z. ges. Expt. Med.*, Vol. 103, pp. 503-538.

DOBRINER, K. (1937). Porphyrin excretion in the faeces in normal and pathological conditions. *J. Biol. Chem.*, Vol. 120, No. 1, pp. 115-27.

DOBRINER K. and RHOADS, C. P. (1938). The quantitative Determination of urinary Coproporphyrin. *Stud. from the Rock. Inst. for Med. Res.*, Vol. III, pp. 409-415.

FOURIE, P. J. J. (1936). The occurrence of congenital porphyrinuria (Pink Tooth) in cattle in South Africa (Swaziland). *Onderstepoort Jnl. of Vet. Sci. and An. Ind.*, Vol. 7, No. 2, pp. 535-566.

FOURIE, P. J. J. and Rimington, C. (1937). *Nature*. Vol. 140., No. 3532, p. 68.

GROTEPASS, W. (1938). *Z. physiol. Chem.*, Vol. 253, pp. 276-280.

MERTENS, E. (1937). Excretion of coproporphyrin III in lead poisoning. *Klin. Woch.*, Vol. 16, pp. 61-62.

RIMINGTON, C. (1936). Some cases of congenital porphyrinuria in cattle: Chemical studies upon the living animals and post mortem material. *Onderstepoort Jnl. of Vet. Sci. and An. Ind.*, Vol. 7, No. 2, pp. 567-609.

RIMINGTON, C. and ROETS, G. C. S. (1937). *Nature*, Vol. 140, No. 3544, p. 584.

RIMINGTON, C., ROETS, G. C. S. and FOURIE, P. J. J. (1938). Quantitative Studies upon porphyrin excretion in congenital porphyrinuria. *Onderstepoort Jnl. of Vet. Sci. and An. Ind.*, Vol. 10, No. 2, pp. 421-429.

RIMINGTON, C. (1937). *Nature*, Vol. 140, No. 3533, p. 105.

RIMINGTON, C. (1938). Porphyrinuria following sulphonilamide: Sulphonilamide dermatitis. *The Lancet*, Vol. 1, No. 14, pp. 770-776.

ROETS, G. C. S. (1938). Coproporphyrin excretion in East Coast Fever of cattle. *Onderstepoort Jnl. of Vet. Sci. and An. Ind.*, Vol. 11, No. 2, pp. 385-388.

SCHUMM, O. (1919). Über weitere üntersuchungen bei Haematoporphyria congenita (II. Mitteilung). *Z. physiol. Chem.*, Vol. 105, pp. 158-172.

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Bovine Congenital Porphyrinuria (Pink Tooth) Inherited as a Recessive Character.

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THE first clinical cases of bovine congenital porphyrinuria were recorded by Fourie (1936). Towards the end of 1935 the Division of Veterinary Services acquired certain grade shorthorn animals from the herd in which the condition was found. These animals were brought to Onderstepoort with the object of studying (1) the hereditary nature of the condition, (2) the pathogenesis, and (3) the biochemical nature of the excreted porphyrins, etc. However, since the condition was recognised in this grade shorthorn herd, three further definite cases of pink tooth in bovines were found in the Union of South Africa as well as a fourth case in a shorthorn bull which is at the present time regarded as a very suspicious case of pink tooth. Confirmation of the diagnosis is being awaited pending the results of certain breeding experiments which were undertaken. These animals fall roughly into four groups, and as such they will be discussed from the hereditary point of view.

Group 1 comprises the Swaziland cases referred to by Fourie, 1936. Group 2 is the suspected shorthorn bull not yet reported in the literature but to which passing reference is made by Fourie and Roets (1934) (elsewhere this journal). Group 3 comprises the Cedara case reported by Fourie and Rimington (1938). Group 4 consists of two Friesland heifers (Ladysmith cases) in which the Government Veterinary Officer, Mr. Flight, made a clinical diagnosis of pink tooth. The diagnosis was confirmed when the urine and faeces were examined spectroscopically and revealed typical porphyrin bands.

GROUP 1.—THE SWAZILAND CASES.

The full history of these cases was given by Fourie (1936). The owner used three shorthorn bulls to improve his herd. When calves were produced as a result of the mating of the third bull to cows and heifers in the herd the first cases of congenital porphyrinuria appeared. The first cases were, therefore, not produced by mating father and daughter. When it was realized that the condition may possibly be transmitted hereditarily, great pains ware taken to find out if the third bull used by the owner was in any way related to

the previous bulls. The owner did not know of any such relationship, but later when careful enquiries were made it was definitely established that the sire of the third bull, and the second bull, were out of the same original herd. The third bull is the one which is at Onderstepoort and is referred to by Fourie (1936) as No. 7015. (See Figure 1.)

Fig. 1.—The porphyrin carrying bull 7015 (Dr.).

The sire which was used in the original herd is a bull named Royal Regent, E.H.B. No. 122649, and it is assumed that this bull, Royal Regent, is the sire of the second bull and that of the sire of the third bull (7015) referred to above. The pedigree of the bull, Royal Regent, is:—

Royal Regent

Rosebud 13th by Rosy Monk 93178 — Royal Stamp
Gd. War Queen by Centaur 70118
G.g.d. Roan Rosebud 3rd by
Pride of Aberdeen 61484
Roan Rosebud 2nd by
Gravesend 46461.

The bull 7015 has normal teeth, his urine does not contain porphyrins such as are present in his descendants, which are clinical cases of congenital porphyrinuria and there is no evidence whatsoever of photo-sensitization. It was, therefore, concluded that if the condition was being transmitted hereditarily, it must be transmitted as a recessive character. Breeding experiments were accordingly planned in order to prove this one way or the other.

Breeding Experiments with Cattle in order to prove that Bovine Congenital Porphyrinuria (Pink Tooth) is transmitted as a Recessive Hereditary Character.

Certain animals which were acquired from Swaziland in December, 1935, formed the nucleus of the animals which were used in the breeding experiments. The animals so obtained are: the bull (No. 7015), two clinically normal cows, being mothers of affected animals; three clinically normal heifers (7019, 7021 and 7022) daughters of the bull and four affected animals (3 steers, 7016, 7017, and 7018 and one heifer, No. 7023—sons and a daughter of the bull, 7015. (Fig. 2.) The breeding experiments were actually carried out in two parts.

Fig. 2.—Females of the porphyrin herd with calves born at Onderstepoort.

In Part 1 the bull 7015 was mated to ten unrelated clinically normal heifers. If the bull is a carrier of the recessive character he will transmit this character to a portion of his progeny. The intention is to mate the bull subsequently to his daughters born out of these unrelated females, in an attempt to produce actual cases of pink tooth. Unfortunately most of the calves produced are males, and the two females are not yet mature, consequently it will take some years before the complete results of this experiment will become available. In the meantime the bull is becoming an old man and I may in the end be forced to complete this part of the breeding experiments with another bull.

However, up to the present eight normal calves were produced by these ten heifers and one calf was aborted as a result of *Trichomonas foetus infection.* All these eight calves are clinically normal and are therefore free from pink tooth. This is regarded as strong albeit not conclusive evidence that the character is not dominant. (Fig. 3.)

Fig. 3.—Unrelated females mated to bull 7015 with calves.

In the second part the bull was mated to all the females acquired from Swaziland. The results obtained are:—

1. *The one old cow* (7024) calved a few days after she arrived here. She herself unfortunately died from acute metritis. The calf, a heifer (7029), is clinically normal and it is practically certain that her sire is the bull 7015 as this was the only bull the owner used at the time. This heifer subsequently was served by her own father and produced a clinically normal male calf (7572). (The Pink Tooth female 7023 is probably a calf of cow 7024—this is according to the owner.)

2. *The other old cow* (7020) has contagious abortion and although served by bull 7015, never produced a calf at Onderstepoort. (The Pink Tooth Ox 7016 is her calf born in Swaziland.)

3. *Cow* 7019. She arrived here as a heifer and her first calf (7025) was actually born in the truck on her way to Onderstepoort. She herself is therefore almost certainly a daughter of bull 7015. One cannot however accept this as an absolute fact, as under practical conditions of farming, it is always possible that a neighbour's bull may sometimes serve a cow, especially on an unfenced farm as is the case with the farm concerned in Swaziland.

Her first calf is a heifer (7025). She is clinically normal. Subsequently she has been repeatedly served by bull 7015, has however never produced a calf and is apparently sterile..

Her second calf is a male (7388), is clinically normal and died when it was about six months old from sand colic. On post mortem examination no evidence of porphyria was present.

Her third calf is also a male (7678), is now six months old and is clinically normal.

This cow therefore produced three calves which are all clinically normal and the probability is that she is not carrying any porphyrin genes.

4. *Cow* 7021 arrived here as a heifer. She is almost certainly a daughter of bull 7015.

Her first calf (7357) is a heifer born on the 7th March, 1937. The calf's teeth were examined within two hours after it was born, when its teeth were seen to be of a pink colour. Its urine, however, was clear, when it was examined on the 9th March, 1937. It became yellowish on standing, but no porphyrin bands were recognized on the 9th March, 1937. It, however, contained a small amount of ether soluble porphyrin. On the 9th April, 1937, i.e. when it was a month old, the urine had a definite pinkish colour. Reference to this case was briefly made by Fourie and Rimington (1937), when it was recorded that the calf passes 7·7 mgm. coproporphyrin per 100 gm. dry weight of faeces and about 40 γ per 100 ml. in the urine. A second heifer calf (7356) born on the same day, and sired by the same bull out of another normal daughter, is normal in every respect. It excretes 0·41 γ coproporphyrin per 100 ml. of urine and but traces of this substance, only recognisable fluoroscopically in the faeces. This is, therefore, the first case of the condition, which I have been able to produce under experimentally controlled conditions. This is a uniformly red calf (see Fig. 4) and did not show any evidence of photosensitization. On account of the great interest which was generally taken in this calf, it was handled rather frequently. The mucous membrane of the mouth may in this way have been injured mechanically, leaving a portal of entry for the necrosis bacillus and when the calf was four months old, it died from calf diphtheria, with which was associated ulcerative and necrotic gastroenteritis and commencing pneumonia. On post mortem examination all the bones were of a reddish pink colour.

Her 2nd calf (7664) was born in April, 1938. This is a clinically normal bull calf.

Her 3rd calf (7904) was born in June, 1939, and is a clinically normal bull calf.

The Cow 7021 is therefore undoubtedly a carrier of the porphyrin gene.

5. *Cow* 7022 arrived here as a heifer. She is almost certainly a daughter of bull 7015. [See Fig. 4(*a*).]

Her first calf (7356) was born here on the 7.3.37, and she is clinically normal.

Her 2nd calf (7710) is a heifer, born on the 18th July, 1938, and is clinically normal.

Her 3rd calf (7941) is a male, born June, 1939.

This calf was examined within 24 hours after it was born and has teeth of an intensive pink colour. The calf is red in colour. This is therefore the second porphyrin calf born out of a clinically normal female. However, the sire of this calf is not bull 7015. It is not

absolutely certain who the sire of this calf is. The eldest of the calves born out of unrelated females and sired by bull 7015 is a bull calf No. 7446, born on the 22.9.37. This bull calf was kept in the same camp as cow 7022 and at the time this cow was served (beginning of September, 1938), to produce the porphyrin calf 7941, the bull calf 7446 was just about 1 year old. There is therefore just a

Fig. 4.—Cow 7021, a carrier (Dr.) with a porphyrin affected calf 7357 (rr.) both mother and daughter sired by bull 7015.

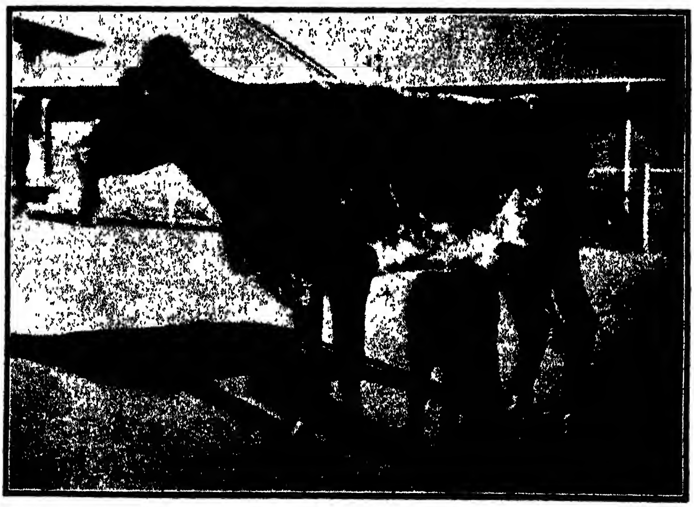


Fig. 4A.—Cow 7022, a carrier (Dr.) with a porphyrin affected calf (7941).

possibility that this bull calf may be the sire of the porphyrin calf 7941. If this is the case, the bull calf 7446 must be a carrier (Dr.) inheriting the porphyrin gene from his sire 7015. It is, however, more likely that the sire is the porphyrin suspect bull 7597 to be referred to later. This bull owing to the marked lesions he develops when exposed to the sun was only allowed to run with these cows during the night, whilst he was stabled during the day. If it is his calf final proof will have been furnished that he is a porphyrin bull, showing marked photosensitization, without well marked discolourisation of the teeth.

After having given birth to two clinically normal calves sired by the carrier bull 7015, it was thought that the cow 7022 may be a completely normal animal (DD), but after the birth of her third calf, she is now definitely classified as a carrier (Dr.).

6. *Cow* 7023.—When she arrived at Onderstepoort she was a young heifer, being the only female showing clinical symptoms of porphyria. She was repeatedly served by the bull 7015, her father, and it was at first thought that she may be sterile. She however eventually held to a service during the late summer (last week in March, 1937), and actually calved on the 30th December, 1937.

The animal 7023 does not show well marked evidence of photosensitization. She is a roan. Therefore she possibly has a good deal of pigment in her skin, this may explain the absence of lesions of photosensitization, together with the fact that the amount of porphyrin excreted by her is considerably less than that excreted by the two worst cases I have, viz. 7017 and 7018.. It is, therefore, possible that although this animal does not show lesions of photosensitization, her photosensitive state, even though this may be of a mild degree, may nevertheless result in a certain amount of interference with normal conception, especially during the time of intense sunshine of midsummer. During the late summer or autumn this is not so severe, and this may have been the reason why she held to a service during that time.

Her 1st and only calf so far was born on the 30th December, 1937. She could not calve normally. I had to deliver the calf. It was a dorso-sacral presentation with lateral deviation of the head. When this was corrected the calf was delivered without difficulty. The tongue was, however, very markedly swoollen and protruding from the mouth and the calf did not breathe. Artificial respiration was practised and eventually breathing commenced. The calf could not stand, it was slung in a bag, with four holes for the legs and fed by bottle. It drank readily, but its head and its neck was never normally straight. It could bear weight on the hind legs, but not on the forelegs. In spite of nursing this calf very carefully it died after ten days. Its inability to stand was put down to an injury of the neck during birth. The teeth of the calf were not markedly discoloured, but on post mortem examination all the bones were found to be of a uniform pink discolouration. This calf, therefore, is the third case of porphyria produced under experimentally controlled conditions.

Evidence of the Recessive Nature of the Inherited Character.

If the character were dominant one would expect clinical evidence of the condition in one of the parents or in a proportion of the first generation progeny of one parent, when mated to an unrelated animal. There is no evidence that this has occurred. When the bull 7015 was mated to ten unrelated heifers the condition was absent in the 8 calves which were born out of these females. It is true that the first cases of the condition appeared in the first generation when the bull was used on the Swaziland farm. If the females to which the bull was mated are unrelated to the bull, one could not exclude the possibility that the character may be partially dominant, but I have not the slightest hesitation in accepting the owner's word that the second bull* he used was out of the same herd as the third bull 7015. From this it is assumed that the bull was being mated to related females.

The evidence which favours the conclusion that the character is recessive is:

(1) The bull transmitting the condition is himself clinically normal.

(2) Under practical farming conditions a proportion of cases appeared when the bull, himself clinically normal, was mated to clinically normal females, which were, however, related to him through a previous common ancestry.

(3) Under experimentally controlled conditions three cases of the condition were produced (1) by mating the bull to his own daughter, she herself being clinically normal, (2) by mating him to another daughter showing clinical evidence of pink tooth, and (3) by mating a daughter (7022) of the bull 7015, to either one of his sons (7446) or to another porphyrin bull 7597.

It is hoped in due course to produce final conclusive proof that the character is a recessive one by mating an affected bull to affected females, when all the progeny must be affected cases. But it will probably take a good many years before this can be done. It will have been observed that no attempt has been made to discuss the percentage incidence of pink tooth in these breeding experiments. Clearly these experiments have not yet reached a stage which would justify a discussion of the percentage incidence. This is of importance in determining whether the character is a simple recessive or otherwise. It is somewhat doubtful, if one would be justified on economic grounds, to maintain sufficient animals, merely to prove this point.

If the character is a simple recessive, the transmission occurs as follows:—D represents the normal gene in so far as the abnormal character is concerned and r represents the abnormal gene. A

* This bull, long since dead, was not a registered animal and therefore no pedigree is available.

completely normal animal in so far as bovine congenital porphyrinuria is concerned will have a genetic constitution of DD, a carrier animal Dr and the affected animal rr. Mating now the carrier bull 7015 (Dr) to a completely normal animal (DD), the possibilities are DD, DD, Dr, Dr. Thus 50 per cent. of the cases will be completely normal and 50 per cent. will be carriers. In mating a carrier animal (Dr) to another carrier animal the possibilities are: DD, Dr, Dr, and rr. Thus 25 per cent. of the progeny will be completely normal, 50 per cent. will be carriers and 25 per cent. will be affected cases.

In mating a normal animal to an affected animal, all the progeny must be carriers (Dr) and when mating affected cases all the progeny must be affected cases (rr).

Up to the time this Swaziland herd was found nothing was known concerning the hereditary nature of congenital porphyrinuria in animals. In referring to congenital porphyrinuria and other anomalies in man Garrod (1923) states that " if the lack of a special enzyme be in each instance the underlying factor, it is to be expected that they should behave as Mendelian recessive characters.

Cockayne (1933) believes one form of congenital porphyrinuria in man to be a recessive disorder due to a single gene, with an unexplained partial limitation to the male sex and another form to be dominant.

The question now arises as to whether this character must be regarded as an inherited lethal character. In accordance with the definition of lethal characters given by Hull (1934) that " all hereditary conditions causing premature death must be classified as lethal regardless of the age at which they are effective ", it seems to me that the recessive inherited character of bovine congenital porphyrinuria must also be regarded as lethal.

It is true that most of the symptoms which develop in bovines are due to photosensitization, when the animals are kept under natural conditions, as a result of which they become exposed to the sun. However, one does meet with cases which do not seem to thrive even though they are protected against the harmful rays of the sun. I have at the moment two oxen (7017 and 7018) under experimental observation. Animal 7017 excretes somewhat more porphyrins than animal 7018. When both are protected against the sun by stabling, animal 7018 puts on weight and looks a magnificent animal; 7017, on the other hand, is not in good condition. It would seem, therefore, that in the case of ox 7017, the presence of the porphyrins may have a general harmful effect. It is, of course, not possible to say that this animal is otherwise completely normal. The animal has however, been tested and found to be negative for tuberculosis and there is no clinical evidence of any other abnormality. Both these animals have at the moment rather puzzling red counts when they were examined haematologically during February, 1938. 7018 had 7·0 million red cells per c.mm., and 7017 had 5·6 millions per c.mm. In August, 1938, 7018 had a count of 5·6 million and 7017 of 5·0 million. In December, 1938, 7018 had 4·4 million and 7017 3·7 million cells per c.mm.

The animals are admittedly being kept under rather abnormal conditions. They do not get any exercise at all and green food is not regularly available. If this may be a factor which is at present influencing the blood picture is not definitely known.

Fourie (1936) pointed out that the cases of congenital bovine porphyrinuria occurs in the male in 77 per cent. of cases. In man also the great majority of cases are males. It would seem as if there may be a partial sex limitation in this condition, but it is certainly not sex linked in the ordinary way, as in South Africa five cases in heifers are known.

Group 2.—Shorthorn Bull 7597.

This bull was briefly referred to by Fourie and Roets (1939). This is an animal showing marked lesions of photosensitization. When the skin lesions were first seen, their cause was not known to the farmer and no attempt was made to protect the animal against the sun. The lesions were therefore of an acute nature. There is marked reddening and in places more or less raw surfaces are exposed. The lesions are very extensive occurring on the skin of the back and extending over large areas of the thorax on each side. (See Fig. 5.)

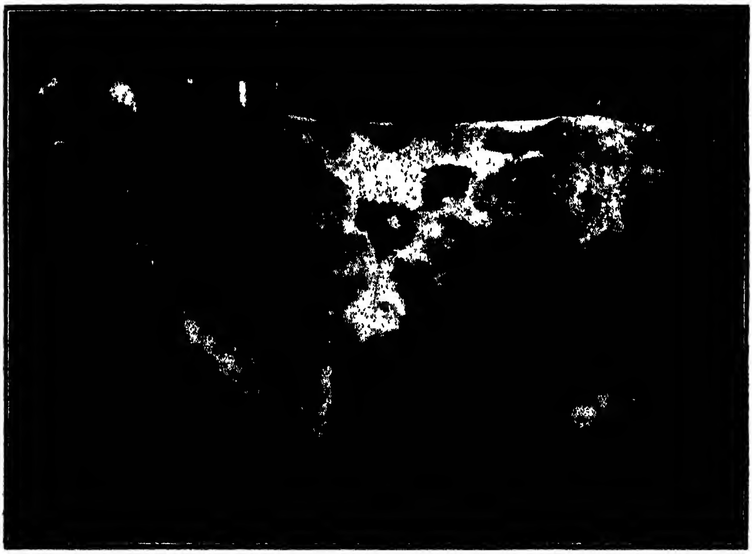


Fig. 5.—The suspected bull 7597 shewing lesions of photosensitization.

The teeth of the animal are, however, not discoloured, or if there is discolouration of the teeth this is so slight that one cannot recognise it definitely when examining the teeth in the ordinary way with the naked eye. Well marked discolouration of the urine is also absent. The porphyrin excretion in the faeces is on a low level, as compared with the affected cases in Group 1, but definitely higher than in other bovines which are clinically normal. [Total daily

porphyrin in cases in Group 1: 7016, ·3 gm.; 7017, 1·6 gm.; 7018, ·8 gm.; bull 7597, 11·298 mgm; clinically normal bovines vary from 3 mgm. to 8·9 mgm., but the animal which has 8·9 mgm. is related to porphyrin animals (see Group 1, No. 7022)]. Uroporphyrin could not be found in the urine and at this stage it is not clear if the absence of the uroporphyrin may explain the absence of discolouration of the teeth and the urine.

When I heard that a certain farmer had this animal showing skin lesions and when I heard that this was a shorthorn bull, it was natural to look for a common ancestry between this animal and the Swaziland shorthorn bull, No. 7015. Knowing that bull 7015 was bred in a herd in which the imported bull Royal Regent was used as a sire, I wrote to the owner of bull 7597 and amongst others I asked him if this bull had any Royal Regent blood in him. He replied that the sire of the bull is Fairview Royal Regent IV. This seemed very satisfactory as it was inferred that "Royal Regent" in the name Fairview Royal Regent IV, must be evidence of some relationship between this bull and the imported bull, Royal Regent. In consulting the herd book in order to confirm this it was immediately seen that the bull, Fairview Royal Regent IV, was sired by another imported bull, Pitsford Abbot 232606. Consequently there is no South African relationship between him and the bull Royal Regent. It was therefore necessary to make a painstaking search for any common ancestry by consulting the shorthorn herd books. The results of this examination are:—

Ancestry of Fairview Royal Regent IV (8367)

Pitsford Abbot 232606
Rickford Raider 175474
Collynie Royal Regent 148043
Masterstroke 126820
Max of Clunie 112487
Douglas Commodore 105256
Jim Sidey 99230 (1907)
Avondale 94085 (1905)

Ballechin Roan Rose by
Victor Chief 82532
g.d. Scottish Rosebud by
Scottish Archer 59893
ggd. Red Rosebud 1st by *Gravesend* 46461
Red Rosebud by Borough Member 33186,
Rosebud by Novelist 34929,
Rosebud 1st (1879) by Diphthong 17681 (1860).

The first common ancestor between the bulls Royal Regent and Fairview Royal Regent IV therefore is the bull Gravesend 46461. If we, however, work backwards from Rosebud 1st we find:

Rosebud 1st is the dam of Rosebud by Sir Christopher.

Rosebud is the dam of Roan Rosebud by Borough Member.

Roan Rosebud is the dam of Roan Rosebud 2nd by Gravesend 46461.

Roan Rosebud 2nd is the dam of Roan Rosebud 3rd by Pride of Aberdeen 61484.

This establishes a further common ancestry between the two bulls. This will be clearly seen if reference is made to the pedigree of the bull Royal Regent already recorded earlier on.

The bull Royal Regent is four generations removed from the bull Gravesend and the bull Fairview Royal Regent is some 12 generations removed from the bull Gravesend and 14 generations from the cow Rosebud the 1st.

It is very interesting to be able to establish this common ancestry and it is of course possible that the recessive porphyrin gene may have been successfully transmitted through all those generations. It is, however, no proof that any of these ancestors were affected cases (rr) or even carriers (Dr) of the porphyrin gene. In a breed like the shorthorn or any pure breed of cattle, it is obvious that one must eventually arrive at a common ancestor for any two animals if one goes back far enough. All that one can say is that this common ancestry may afford a possible clue of the manner in which the porphyrin gene has been transmitted. In this connection one should also bear in mind that there is no definite evidence that the bull Royal Regent 122649 is a carrier (Dr.) of the porphyrin gene. He is implicated by inference, as the porphyrin carrier bull 7015 and the bull which was previously used in the Swaziland herd were out of the same original herd in which the bull Royal Regent was used as a sire.

If the bull Royal Regent and the bull Fairview Royal Regent IV actually did inherit the recessive gene from a remote common ancestor one is here faced with a most extraordinary coincidence in that this rare anomaly was introduced into South Africa along two separate but related blood lines and that in the case of the Royal Regent blood line clinical cases appeared as a result of accidental close breeding. The dam of bull 7597 is not a registered cow and up to the present I have not been able to find out where she may have obtained the recessive character.

" Royal Regent " in the name Fairview Royal Regent IV was used because of the relationship of the bull to its ancestor Collynie Royal Regent—but " Royal Regent " in Collynie Royal Regent, I am told is pure coincidence and was not deliberately used to indicate a relationship between the bulls Royal Regent and Collynie Royal Regent.

GROUPS 3 AND 4. THE CEDARA CASE AND THE LADYSMITH CASES.

The Cedara case was described by Fourie and Rimington (1938). Flight (1938) described the occurrence of the condition in two heifers (Ladysmith cases). I am indebted to him for a personal communication concerning their history. A clinically normal registered Friesland Bull Kamnatie Charles was used as a sire in the Ladysmith area for a number of years. He sometimes served his own daughters. The two affected heifers are his granddaughters, out of his own daughters. Both the dams are clinically normal. Those of his other descendants which were available for examination were found to be clinically normal. A full sister of one of the affected heifers is also clinically normal. These cases constitute further evidence of the recessive nature of the character. (See Fig. 6.)

Fig. 6.—The Cedara case (rr.) with well grown calf (Dr.).

It would be surprising if one cannot establish a common ancestry, by a careful study of the ancestors of the Cedara case and that of the Ladysmith cases as both these are Frieslands. This indeed is the case, and here also, as in the case of the two shorthorn groups, the common ancestry may possibly indicate how the recessive character has been transmitted, but one cannot accept it as proof that that is the case. The common ancestor is the bull Albert 1306^{H}. He is five generations removed from the Cedara case (rr) and four generations from the carrier bull Kamnatie Charles (Dr) both on the sire and the dam side of the dam (De Goede Laaste) of the bull Kamnatie Charles.

Alberta in the name of the bull Alberta Roland does not necessarily indicate Albert 1306^{H} blood. Alberta is merely a prefix used by a particular breeder. This common ancestor may or may not contain the recessive porphyrin carrying gene. Should one get

other cases in which the Albert blood shows up so prominently one would have reasonable grounds for suspecting it as the carrier of this abnormal factor.

The ancestry of the Cedara case is:—

Cedara Ria 4th No. 177—Cedara case—

Cedara Ria 2nd No. 113 — Cedara Joe

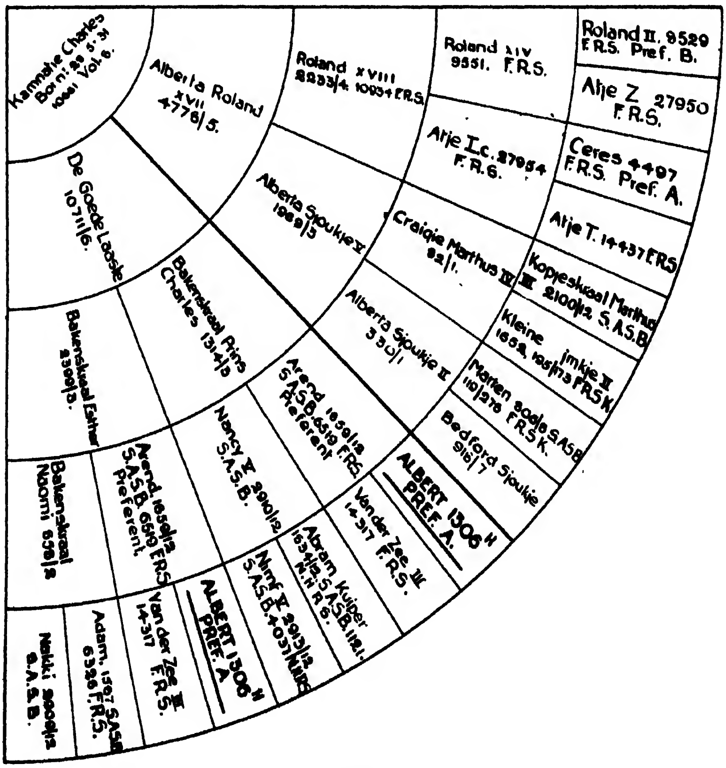
Cedara Jetzke 2nd — Nelsrust Commodore 2nd 1707

Ogden Hall *Alberta* — Nelsrust Beatty 2nd

Akke III 1902 119/266 F.R.S.K. — Albert II 1247

Akke II — Albert 1306[H]

The extended pedigree of the bull Kammatie Charles, kindly supplied by the Secretary of the Friesland Breeders Association, Bloemfontein, is:—



At the present time there is this extraordinary fact, that bovine congenital porphyrinuria has been found in four different groups of cattle in South Africa. (For the moment I am assuming that bull 7597 is a genuine case of the condition In the absence of reliable standards of quantitative porphyrin excretion in a sufficient number of normal animals, the final proof must be furnished by breeding experiments, already commenced, unless, of course, this bull should prove to be sterile, an eventuality which is not unlikely.) Two of the groups are shorthorns, in which it has been possible to establish a common ancestry, albeit a remote one. The other two groups are Frieslands and here a common ancestry appears within the first five generations. Although this is the case, a conclusion at this stage that the common ancestor is the carrier of the porphyrin gene in the groups of shorthorns and Frieslands concerned is premature.

It is, however, very remarkable that so many of these cases should be found in South Africa and not in the parent stock in Great Britain and Holland. My own opinion is that there are certain to be cases in those countries, but they remain unrecognised. By saying this I do not mean to convey that veterinarians in South Africa are better or more accurate observers than their colleagues in Great Britain and Holland, but the reason almost certainly is to be found in the difference of climate in South Africa and that of Holland and Great Britain. In South Africa the intense sunshine throughout the year is responsible for marked lesions which develop because of the photosensitization in the porphyrin animal, and also for the fact that such animals do not thrive. Such abnormalities force the owner to seek professional advice, whereas in Europe, the lack of intense sunshine will not produce lesions and disturbances to the extent they are seen in South Africa and the cases may, therefore, never be brought to the notice of veterinarians in those countries.

Summary and Conclusions.

1. In mating a bull (7015), being a carrier of the porphyrin gene (Dr) to ten unrelated heifers, 8 calves were produced. These are all clinically normal. This is regarded as evidence that the inherited character is not dominant.

2. In mating bull 7015 (Dr) to his own daughters the results are (*a*) 7019 had 3 normal calves. 7019 is therefore probably a DD.

(*b*) 7021 had 2 calves. The first calf suffered from congenital porphyrinuria. The second calf is normal. She is therefore a carrier (Dr).

(*c*) 7022 had 3 calves, two are normal, the 3rd is a case of pinktooth. She is therefore undoubtedly a carrier (Dr).

(*d*) 7023 is an affected heifer (rr). She had one calf, also an affected case.

(*e*) 7024 is not a daughter, but related to bull 7015 through her own sire. She had one affected calf, 7023, and one normal calf 7029. This normal calf was mated to her own father (bull 7015) and a normal calf was produced. 7024 is therefore a carrier (Dr) and her calf 7029 may be either completely normal (DD) or a carrier (Dr).

These results indicate that bovine congenital porphyrinuria is inherited as a recessive character.

3. The recessive character was probably introduced into the Swaziland herd by bull No. 2 used by the owner. This bull was apparently never used on this own daughters and as the character is not dominant, no cases were seen in that herd during the time of his reign. The 3rd bull, No. 7015 was bred out of the same herd as bull No. 2. They were therefore related and probably inherited the recessive character from the same sire, the bull Royal Regent. Bull 7015, was therefore mated to related females and within a relatively short time, 13 cases of the condition were observed in that herd, whilst the bull 7015 was being used as a sire.

(4) When the Friesland bull Kamnatie Charles was inbred to his own daughters two cases of the condition were observed. This bull is therefore a carrier (Dr). He is related to a grade Friesland cow, suffering from the condition (rr) (Cedara Case).

REFERENCES.

COCKAYNE, E. A. (1935). Inherited abnormalities of the skin and its appendages, pp. 105 and 106.

FOURIE, P. J. J. (1936). The occurrence of congenital porphyrinuria (Pink Tooth) in cattle in South Africa (Swaziland). *Onderstepoort Jnl. of Vet. Sc. and An. Ind.* Vol. 7 No. 2, pp. 535-565.

FOURIE, P. J. J. and RIMINGTON, C. (1937). Living animal cases of congenital porphyrinuria. *Nature.* Vol. 140, No. 3532, p. 68.

FOURIE, P. J. and RIMINGTON, C. R. (1938). A further case of congenital porphyrinuria (Pink Tooth) in a living grade Friesland Cow in South Africa (Cedara) case. *Onderstepoort Jnl. of Vet. Sc. and An. Ind.* Vol. 10, No. 2, pp. 431-436.

FOURIE, P. J. J. and ROETS, G. C. S. (1939). Quantitative studies upon Porphyrin excretion in bovine Congenital Porphyrinuria (Pink Tooth) No. 2. *O.P. Jnl. of Vet. Sc. and An Ind.* (1939). Vol. 13, Nos. 1 and 2.

GARROD, A. E. (1923). Inborn errors of metabolism. p. 29.

HUTT, F. B. (1934). Inherited lethal characters in Domestic Animals. *Cornell Veterinarian.* Vol. 24, No. 1.

Section VIII.

Wool Research.

BOSMAN, V. AND VAN WYK, C. M.	Notes on the determination of the fibre fineness of a Merino wool staple.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 13, Number 2, October, 1939.
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Notes on the Determination of the Fibre Fineness of a Merino Wool Sample.

By V. BOSMAN and C. M. VAN WYK, Section of Wool Research, Onderstepoort.

THE technique adopted in South Africa for determining the fibre fineness of a sample of wool, although essentially that described by Duerden (1929), has certain modifications which are here outlined.

In the present contribution it is intended to deal with the method of preparing a wool sample for mounting and with its measurement. The results dealing with the sampling of the merino fleece, both on and off the live sheep, will be dealt with in another paper.

PREPARATION OF THE SAMPLE FOR MOUNTING.

(*a*) *Preliminary degreasing of sample.* The greasy sample is washed in cold benzene so as to remove most of the impurities. The ether method of mixing is subsequently used and this removes the residual impurities.

(*b*) *The cutting of the fibres for mixing.*—Owing to the variability in fibre fineness along the length of fibres, it is necessary that an average of several points along the fibre bundle be taken, for which purpose the method described by Duerden (1929) is being used. It consists in either cutting the sample into fragments along the entire length of the fibre bundle, or else, removing the fragments at equal intervals of approximately a ½ inch along the length. Malan, Carter and van Wyk (1938) showed that there was no difference between the two methods in the case of a small sample which was cut at three points and also along its entire length.

Methods for cutting the fragments have also been investigated and those of other workers tested. Wildman (1936), Wildman and Daniels (1937) and later Malan, Carter and van Wyk (1938) gripped the fibre bundles between strips of paper. Huberty (1938) described a method of cutting fragments with a razor blade, a method which it is claimed, results in shorter and more uniform fragments. This method was tested and it was found difficult to cut, with a single

stroke, all the fragments from one region, an essential condition in the case of raw wool, as will be clear from the following considerations.

It has been shown (Bosman, 1937), that such factors as differences in the plane of nutrition of the sheep, period of lactation of the ewe, etc., can cause marked differences in the fibre fineness of different regions of the staple. Where fragments are therefore removed at equal intervals from the staple, care is necessary to ensure that all regions contribute the same number of fragments to the total.

After the fragments have been cut at intervals, they can quite easily be clipped into shorter pieces with the aid of a pair of finely pointed scissors, but for raw wool this method has the disadvantage that the originally cut longer fragments probably contribute a greater proportion of smaller fragments to the total than do the shorter original fragments, and, if longer fragments are cut in any special region, the result is biassed in favour of that region. If, with Huberty's method it is not possible to cut the ultimate fragments for mounting with a single stroke of the razor blade so as to ensure an equal number of fragments from each region, the method is not suitable for raw wool.

In routine practice, the method of gripping the wool bundle between the fore-finger and thumb has given very good results and the method has been adopted for general use in our laboratories. With practice, and the necessary care, routine assistants are able to use this method to better advantage than has been the case with other methods. It has also been found that uniform short fragments give a better mixture than do the uneven longer ones and very good results have been obtained with a little training. Slides made by this method have shown an even fibre distribution without any fibre clusters on the slide.

There is a limit to the shortness of cutting the length of fibre fragments, since the scissors distort the ends of the fragments. It has been found that fibre lengths of from $\frac{1}{3}$ to $\frac{1}{2}$ millimeter can be cut with ease and give satisfactory results.

The objection to this method, that several fragments from the same fibre are included and consequently that the same fibre may be measured more often than once, has been investigated. It was found that the bundle contains many thousands of fibres while the number of points at which fragments are taken seldom exceeds 10, so that the chance of measuring the same fibre more often than once is exceedingly small.

Mounting the Fragments for Measurement.

The cut fragments are transferred to a test tube of about 2 cm. diameter and 6 cm. deep containing ether. The fibres are shaken, giving a uniform dispersion of fragments which are allowed to settle. Successive clumps, taken at random by finely pointed forceps are placed in rows on a black cardboard where the ether is allowed to evaporate.

A thin layer of Euparal mounting medium* (which has been found to be superior to other mounting media such as Canada Balsam, Cedar wood oil, etc.), is spread on a glass slide and the clumps of fragments, taken at random from the cardboard, stirred by the aid of dissecting needles, into the Euparal until the fragments are well distributed.

A cover slip is finally pressed down over the slide, excess Euparal being removed at the edges of the cover slip by means of blotting paper. A few fragments of fibres are removed with the excess Euparal, but the remainder is sufficiently representative of the original. With practice it is possible to use just the right amount of Euparal for a minimum loss of fibre fragments and also for a complete absence of air bubbles in the slide.

The question as to whether successive clumps of fragments taken from the test tube are similar, has been tested. Each successive clump that was placed on the cardboard, was mounted on a slide and measurements taken. The results are summarised in Tables 1 and 2.

TABLE 1.

The Fibre Fineness of successive Clumps taken from the Ether Test Tube.

Clump No.	Mean Fibre Fineness. μ
1	17·45
2	17·55
3	17·76
4	17·69
5	17·52
6	17·81
7	17·68
8	17·97
9	17·51
10	17·32
20	18·13
30	18·11
40	17·98
50	17·97

TABLE 2.

Variance.	Standard Dev.	Log$_e$ S.D.	Z.
Between slides	2·0215	·7041	·2659 (n_1 = 13)
Within slides	1·5503	·4382	(n_2 = 3486)

* The Technical Committee of the International Wool Textile Organisation at its 1939 meeting decided that for routine purposes not requiring the preparation of permanent slides, cedarwood oil was preferable.

This aspect has, however, been carefully investigated in our laboratories and it has been found that for studies of wool from production aspects, such as in breeding experiments and others, where permanent slides for reference purposes are essential, the use of Euparal is preferred. Furthermore, cedarwood oil does not give the same clear fibre outline as Euparal.

According to Fisher, the analysis of variance indicates no significant difference between the variances between and within slides. The fact that several clumps, taken at random, made up one slide, justifies this method of preparing slides.

An alternative method of mounting fibres was described by Malan, Carter and van Wyk (1938). The fragments were mixed in ether in a shallow dish, excess ether was poured off and the remaining ether allowed to evaporate. Clumps of dry fibres were shaken out by tapping the cut fragments over the slide that had a thin layer of Euparal. Another thin layer of Euparal was then placed on the cover slip which was pressed down over the slide. It was found that, by this method, fragments of fibres were prevented from being forced out with excess of Euparal.

While this method gave satisfactory results, it has not found favour in our laboratories, the reason being that dry fibres are difficult to handle this way, and there is a possibility that the lighter, finer fibres may be blown away during the tapping process.

Humidity.

All slides are mounted in a room maintained at constant humidity and temperature, and in this way variations due to swelling of the fibres are eliminated. Tests were, however, made for conditions outside the Constant Humidity Chamber, so as to be applicable for cases where the controlled conditions were not available. The test, carried out over a period of 19 days, consisted of cutting up a sample of wool into fragments and mixing in ether. Clumps of fragments were placed on a black cardboard and dried in a desiccator for a few days. The clumps of fibres were then placed in a room where the relative humidity was determined at intervals by a whirling hygrometer. Two slides were prepared from the fragments at different humidities and, as a check on the moisture content, a standard sample, which had been washed in ether, was weighed in proximity to the fragments. The results are given in Table 3.

TABLE 3.

Relative Humidity.	Temperature.	Mean Fineness (μ).	Regain of Standard Sample.
Per cent.			Per cent.
13	32° C.	20·4	6·8
18	27° C.	20·2	8·6
30	29° C.	20·0	9·6
60	25° C.	20·5	12·8
70	21° C.	20·6	16·2
76	22° C.	20·8	16·9
80	22° C.	21·0	17·9

The difference between the values at 13 per cent. and 18 per cent. R.H. is not significant but the difference between the values at 13 per cent. and 30 per cent. R.H. is just significant at the 5 per cent. probability level. Such irregularities may be ascribed to

several causes, such as the large variations in temperature, the fact that some humidities were reached under adsorption conditions and others under desorption conditions, and the lag in taking up or losing moisture at each humidity. The results show the necessity for preparing the slides under controlled conditions.

The Influence of Ether.

It has been stated by Daniels (1938) that the values obtained from permanent slides made from ether and Euparal were consistently lower than those made from cedar oil, and this was attributed to the contraction of the fibres by ether.

A series of tests, using different treatments and mounting media, was therefore carried out.

A sample was cut into fragments which were mixed in ether. Fibre clumps were withdrawn from the mixture and laid on a black cardboard in the usual way. When the ether had evaporated, every second clump was placed in a test tube containing distilled water. The fragments were mixed and shaken in water until all had been thoroughly wetted. Clumps were then withdrawn and laid on a card. Both sets of clumps, the ones mixed in ether only and the ones mixed in ether and water, were placed in a desiccator for 24 hours and then exposed to an atmosphere of constant humidity and temperature. Slides were made of each set of clumps, using Euparal, for the measurement of 500 fibres from each set. This procedure was followed with two samples, the results being summarised in Table 4.

Table 4.

Sample.	Mean Fineness (μ).		Difference (μ).	S.E. of Diff. (μ).	"t" = Diff./S.E. of Diff.
	Mixed in Ether.	Mixed in Ether and washed in Water.			
1................	20·012	19·732	0·280	± 0·2385	1·174
2................	17·292	17·152	0·140	0·1852	0·756

The values obtained from fibres mixed in ether are not significantly different from those mixed in ether and water (when $n = 500$).

A further series of tests based on 15 pairs of slides, in which some fragments had been mixed in ether only and some in water only, was carried out. The results are summarised in Table 5.

Table 5.

Mean Fibre fineness of slides. μ		Difference.	S.E. of Diff.	"t".
Mixed in Ether.	Mixed in Water.			
20·696	20·411	0·285	± 0·075	3·80

These results show a significant difference between the fibres mixed in ether and those mixed in water. It is shown that the fibres mixed in ether are 1 per cent. thicker than those mixed in water and it appears that the ether caused the fibres to swell.

Tests were also made on fibres mounted in cedar wood oil and those mounted in Euparal. The results are given in Table 6.

TABLE 6.

Treatment.	Mean Fibre Fineness (μ).		Difference.	S.E. of Difference.	"t".
	Mounted in Euparal.	Mounted in Cedar Wood Oil.			
Mixed in ether....	20·696	20·406	0·290	$\pm$ 0·1201	2·84
Mixed in water....	20·411	20·281	0·130	$\pm$ 0·0576	2·26

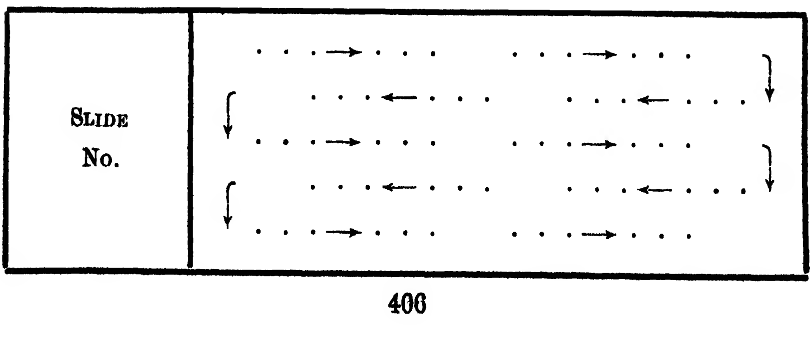
In both cases the fibres mounted in Euparal gave significantly higher values than those mounted in Cedar wood oil, the difference again being of the order of 1 per cent. These figures also suggest that, when slides are made with fibres mixed in ether and mounted in Euparal, the results are about 2 per cent. higher than those mixed in water and mounted in Cedar wood oil. In all these tests the fibres were dried in a desiccator before being exposed to the constant humidity conditions, so that swelling took place under conditions of adsorption.

THE MEASUREMENT OF THE FIBRES.

The measurements were made on Zeiss Lanameters. The magnification on the screen of each instrument was found to vary from the centre outwards and it was necessary to use only an area that could be accurately standardised. A circle of radius 4 cm. was drawn in the centre of the screen, the magnification of fibres falling within this circle being adjusted to exactly 500. In routine measurements only fibres lying within this circle are measured.

Each slide containing the wool fragments is systematically read in the manner shown in Figure A. Dotted lines represent the regions where fibres are measured while arrows indicate the direction in which the slide is moved.

FIG. A.



There are ten regions of measurement and one tenth of the desired number of readings are taken in each region. By this method measurements are made over the whole slide.

As a check on the uniformity of the distribution of the fragments over the slide, the results are recorded in successive groups of 25 measurements.

An analysis of variance is made comparing the variance between groups of 25 with the variance within groups of 25. Cases which occurred in a sampling experiment during the measurement of 10 slides are shown in Table 7.

TABLE 7.

Comparison of Variance between and within Groups of 25 Readings.

Variance.	D.F.	CASE 1. Mean Square.	CASE 2. Mean Square.	CASE 3. Mean Square.	CASE 4. Mean Square.	CASE 5. Mean Square.
Between groups of 25 readings..	99	4·8411	4·1002	3·6678	4·0997	4·5157
Within groups of 25 readings...	2,400	4·3529	4·1076	4·3512	3·9925	4·2757
TOTAL........... ..	2,499	4·3722	4·1073	4·3241	3·9967	4·2852

These results show that the fineness distribution of the fragments over the slide is satisfactory.

In routine practice, duplicate slides are usually made and half the total number of readings taken from each slide. The difference between the slides has to conform to the demands of statistical theory.

THE NUMBER OF FIBRES MEASURED.

Since the number of measurements necessary from any one sample depends on the variability within the sample, the statistical constants serve as a guide for determining the number of measurements to be made. It has been shown that for Merino wool, 250 measurements are generally sufficient to bring the permissible error to within 3 per cent. of the mean and although many of the samples need less than 250 measurements, it is simpler and quicker to measure 250 straight off than to measure a number less than this for a preliminary statistical test.

The procedure thus is to measure 250 fibres on all Merino samples and then work out the statistical constants. It is very seldom that more than this number need to be measured.

A convenient chart (Fig. B) showing the relationships between the statistical constants and the number of fibres necessary for measurement is used and has proved very valuable for routine work.

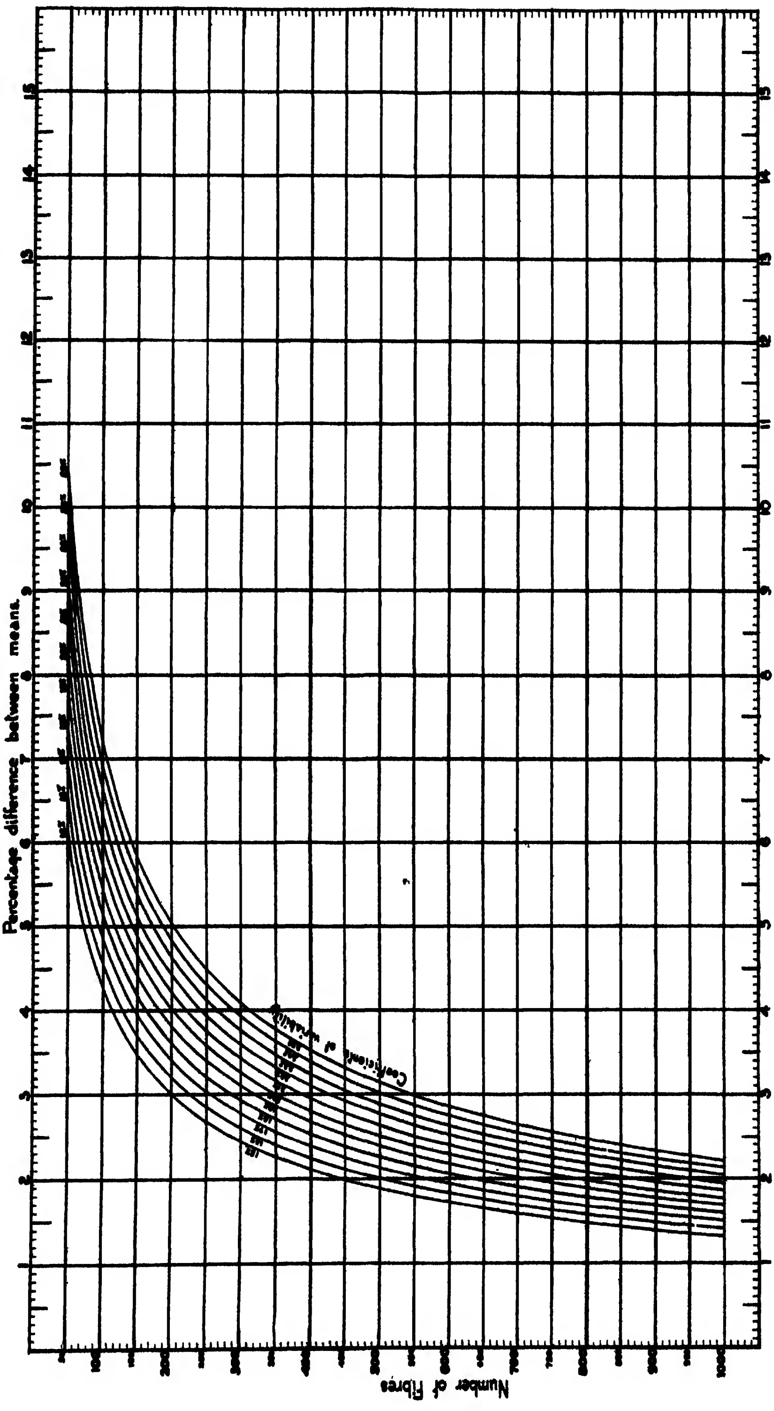


Fig. B.—The relationships between the statistical constants and the number of fibres necessary for the measurement of a sample of merino wool. The values are given for a 5 per cent. probability level of significance.

THE HUMAN ELEMENT AMONG OBSERVERS.

Serious differences have been noticed among different observers, and special investigations on this aspect have been undertaken in our laboratories. It has been observed that although differences among observers are often constant, they are not necessarily always so.

Three sets of slides measured by four observers are here summarised, each observer measuring 125 fibres from each slide.

The results are given in Tables 8 and 9.

TABLE 8.

Mean Fibre Fineness (μ).

	Observers.			
	A.	B.	C.	D.
1st Set (26 slides)......................	18·84	18·46	18·76	18·69
2nd Set (43 slides)......................	27·14	26·67	27·31	27·04
3rd Set (36 slides)......................	23·62	22·82	23·92	23·65

TABLE 9.

Differences between Observers A, B, C and D (in μ).

	B.	C.	D.
A. 1st Set...............	**0·38** ± 0·0907	0·08 ± 0·0661	0·15 ± 0·0765
A. 2nd Set...............	**0·47** ± 0·0942	−0·17 ± 0·1194	0·10 ± 0·1242
A. 3rd Set...............	**0·80** ± 0·0847	−**0·30** ± 0·0599	−0·03 ± 0·0533
B. 1st Set...............		−**0·30** ± 0·0676	**0·23** ± 0·0769
B. 2nd Set...............		−**0·64** ± 0·1263	−**0·37** ± 0·1280
B. 3rd Set...............		−**1·10** ± 0·0954	−**0·83** ± 0·0794
C. 1st Set...............			0·07 ± 0·0496
C. 2nd Set...............			**0·27** ± 0·0921
C. 3rd Set...............			**0·27** ± 0·0450

Significant differences are in black type.

The results show that there are significant differences between observers except between A and D, where the differences are not significant. It is also shown that the differences are not constant.

An important fact, that was also established, is that, when slides are measured continuously without the observers having a change on to some other work and without a periodic check on the observers,

serious differences can develop. It is shown in the tables that, in general, there is a tendency for difference between observers to be greater in the third set of slides except between observers A and D. A closer study of the results has led to the conclusion that a lack of concentration developed during the reading of the third set. This was largely attributed to the factor of fatigue.

Special tests were also made on the systematic differences between observers. A hundred synthetic fibres were mounted parallel across the slide and the four observers were made to measure the hundred fibres carefully at the same spots. The averages of three such sets of readings, each taken at a different level of the slide, are summarised in Table 10.

TABLE 10.

Differences between Observers on Standard Slides (in μ).

	B.	C.	D.
A.	+ 0·26 0 − 0·15 } 0·04	0·04 − 0·14 − 0·27 } −0·12	+ 0·28 + 0·06 − 0·02 } +0·11
B.		− 0·22 − 0·14 − 0·12 } −0·16	− 0·02 + 0·06 + 0·13 } +0·06
C.			+ 0·20 + 0·20 + 0·25 } +0·22

The results of Table 10 show that the differences between observers were small and insignificant and a closer study of the observers showed that differences to a large degree depended on the fact that, when the edges of the fibres, presented for measurement, fell midway between the scale divisions on the lanameter, judgment as to which division the measurement belonged, differed among individuals. Such differences may possibly be reduced by the use of smaller scale divisions and larger magnifications, but then in the case of Merino wool this would tend to make measurements more difficult because of the serrated edge of the wool fibres.

In order to minimise differences between observers in our department dealing with fibre fineness measurements, a system was introduced whereby all measurements were made by pairs of observers, one of the pair taking half the required number of readings on a slide, while the other enters the readings, the pairs being chosen in such a way that the averages of the measurements of each individual of the pair counterbalances differences between the pair It was found that the effect of the factor of fatigue was considerably reduced by each pair measuring the 125 readings in turn. Frequent intervals of rest are also prescribed in the form of changes to some other type of work.

The results now obtained from different pairs of assistants are very satisfactory, differences among observers being reduced to a minimum.

The results of 42 slides are given in Table 11.

TABLE 11.

Differences between Observers—Averages of 42 Slides (in μ).

	B.	C.	D.
A.	− 0·030 ± 0·0737	− 0·015 ± 0·033	+ 0·093 ± 0·0657
B.		+ 0·015 ± 0·0691	+ 0·123 ± 0·0879
C.			+ 0·108 ± 0·0693

These differences are below the possible error due to sampling. The system whereby pairs of observers take measurements of the same slide serves as a check on the observers and a system of entry of the results has been instituted whereby the results can be summarised at a glance.

In addition, duplicate slides are usually made of the same lot of fragments and it was found that, when one observer of a pair makes half the required number of measurements from one slide and the other observer the remainder on the other slide, the making of the slides as well as the observers are under control.

THE WEIGHT-LENGTH METHOD OF DETERMINING FIBRE FINENESS.

It has been suggested (Wilsdon, 1938) that in cases where errors are likely to occur between the measurements of different observers, the weight-length method should be used for arbitration purposes.

A comprehensive set of tests was carried out on samples of Merino wool for ascertaining the relative merits of the weight-length method and the projection or microscope method.

The following conclusions were arrived at:—

1. The weight-length method depends on determinations of fibre length as straight fibre length. In this respect tests have shown that different observers obtain different results on the same sample of wool and even on the same fibres, due to a personal element. Because of this the weight-length method cannot be said to be more reliable than the projection method.

2. In the weight-length method (Roberts, 1930) it is assumed that the specific gravity of all wool is constant at 1·30. It has, however, been found that there is a variation of 1·3 per cent, in the specific gravity of South African wool (van Wyk and Nel, 1939). On a Merino wool, therefore, with a fibre fineness of 20μ, errors of up to 0·14μ may accrue from the assumption that all wool has the

same specific gravity. The weight-length method is, therefore, at a disadvantage unless the specific gravity of each sample analysed, is also determined. This, however, is impracticable.

3. All wool samples do not adsorb moisture to the same degree (van Wyk, 1939) hence the use of a standard sample for dry weight determinations will give rise to error.

4. The weight-length method gives a weight-biassed result (Roberts, 1930) while the microscopic or projection method gives a length-biassed result (Wildman, 1936) and it has been found that the two methods do not give the same measurement.

The weight-length method is based on the dry fineness of the fibres when the dry weight is obtained. Determinations on 56 Merino wool samples by the two methods showed that the microscopic method gave values which were 5 per cent. higher than those obtained by the weight-length method, the latter being on the dry weight basis. This difference is presumably due to the swelling of the fibres with adsorption of moisture.

If the weight used is based on a certain humidity and temperature, errors will be introduced by differences in the specific gravity, moisture adsorption and swelling of the different types of wool, factors which have to be controlled where extreme accuracy is needed.

5. In the case of Merino wool the average weight of a fibre from a 3-inch staple is about ·04 milligrams. This necessitates the counting of a large number of fibres for the weight-length determinations, to ensure any degree of accuracy in the weighing.

Summary.

The technique for determining the fibre fineness of a sample of wool as used in South Africa is outlined.

The methods of cutting the fibre bundle into fragments and the mounting of these on a slide are described.

The effect of relative humidity during the preparation of the slide shows the necessity for controlled moisture and temperature conditions.

The effect of the ether used for mixing the wool fragments and the Euparal mounting medium are described.

The method of measurement, the system of recording the results and the number of fibres to be measured in Merino wool are recorded.

The question of the personal errors of different observers has been studied. The differences between observers are not necessarily constant, the factor of fatigue being of special importance. Methods for reducing these errors to a minimum are described.

The weight-length method for determining fibre fineness, suggested for arbitration purposes, is discussed and difficulties peculiar to this method are enumerated.

REFERENCES.

BOSMAN, V. (1937). Biological studies on South African Merino wool production. *J. Text. Inst.* XXVIII, 8, pp. P.270-P.306 and 9. pp. P.321-P.353.

DANIELS, H. E. (1938). Note on variations in the standard method for fineness measurement. *Proc. Tech. Committee Intern. Wool Text. Org.*, London, 1938.

DUERDEN, J. E. (1929). Wool research in South Africa. *Papers for Pan-African Agricultural Conference.* Part III, Govt. Printer, Pretoria, pp. 34-50.

HUBERTY, A. (1938). Méthodes de préparation des plaques de fibres de laines pour les analyses de finesse effectuées sur le même ruban ou groupe de rubans constituant l'échantillon. Dispersion comparée des résultats. *Proc. Tech. Com. Intern. Wool Text. Org.*. London (1938).

MALAN, A. P., CARTER, H. B., AND WYK, C. M. (1938). Wool studies III. The uniformity of a series of fibre thickness measurements on a small sample of medium merino wool. *Ond. Journ. Vet. Sci. and An. Ind.*, Vol. 10. No. 2. pp. 445-466.

ROBERTS, J. F. (1930). Fleece analysis for biological and agricultural purposes. The average fineness of a sample of wool. *J. Text. Inst.* XXI. Vol. 4. pp. T.127-T164.

VAN WYK, C. M. AND NEL, H. A. M. (1939). A preliminary note on the specific gravity of South African Merino wool. (*Communication to the International Wool Textile Organisation*. Brussels.)

VAN WYK, C. M. (1939). A note on the water adsorption by South African Merino wool. (*Communication to the International Wool Textile Organization*. Brussels.)

WILDMAN, A. B. (1936). Some characteristics which enter into the assessment of wool quality, and their estimation in the fleece. *J. Text. Inst.* XXVII. pp P.181-P.193.

WILDMAN, A. B. AND DANIELS, H. E. (1937). A comparative examination of methods of analysis of wool for fibre diameter and length, Part II. The analysis of raw wool for fibre fineness. *J. Text. Inst.* XXVIII. Vol. 6. pp. T.202-T.205.

WILSDON, B. H. (1938). Minutes of meeting of technical committee of the International Wool Org. Item II b, 13th June, 1938.

Section IX.

Anatomy-Teratology.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 13, *Number* 2, *October*, 1939.

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Prenatal Growth in the Merino Sheep.*

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CONTENTS.

* A Thesis accepted by the University of South Africa for the degree of Doctor of Veterinary Science.

CHAPTER 1.—INTRODUCTION.

OBJECT OF WORK.

THAT growth during the foetal period does not differ qualitatively from that subsequent to birth is the view generally accepted. It is maintained that, in spite of its greatly varying rates and its change of direction from progressive during the developmental period to regressive during senescence, growth is one continuous process starting with fertilization of the ovum and ceasing with death of the individual. The progressive part of the cycle is " divided by the incident of birth into pre- and postnatal periods " (Arey, 1931). When it is considered how readily the latter period lends itself to investigation, it is not surprising to find that much work has been carried out in this connection. Reference to Brody *et al* (1926) or to Hammond (1932) will give an idea of the extent of the available literature.

In direct contrast to this wealth of material is the paucity of information regarding the prenatal phase of development. What knowledge does exist has been gained in studies mostly on small laboratory animals, such as the rat (Stotsenberg, 1915), the mouse (MacDowell *et al*, 1927), and the guinea-pig (Draper, 1920). In general the results of this work have confirmed the thesis of continuity of the growth curve. However, MacDowell and his associates believe that on account of qualitative differences in growth during the earliest stages, age for the purpose of growth curves should be calculated from the time of formation of the " embryo proper ".

For man and the domestic mammals there is a lack of extensive and reliable data. That such work on the human being presents well nigh insurmountable difficulties is only too readily understood. A brief indication of the complexity of the problem is given by Mall (1910). Not being bound by the same ethical laws as his medical colleague, the worker in the veterinary field encounters but one major obstacle—the brake of economic considerations. To expand on the seriousness of this problem would merely be labouring the point. Therefore, it is apparent that any work which aims at making available additional material advances greatly the facilities for the investigation of prenatal growth.

Scattered throughout the veterinary literature are isolated references to weights and dimensions of small numbers of foetuses of known or assumed ages. Due to the diversity of conditions under which the observations were made and the almost general lack of accurate definition of procedure, these figures cannot be employed in the compilation of one comprehensive table.

In this field most workers have avoided committing themselves as to the age of their material. Invariably they have relied upon either length or weight as an indication of age-sequence. Consideration of breed and individual variability will show that such criteria are apt to give misleading results. Especially would this be the case where the material is obtained from such an

uncontrolled source as a public abattoir. A further disadvantage of this procedure, exposed by Lowrey (1911), is that in the absence of data regarding age, absolute growth-rates cannot be given. The worker has to content himself with expressing growth of systems and organs relative to body weight.

The existence of adequate ageing standards would make available for studies on prenatal growth the abundance of material always obtainable at large abattoirs. As a result of the work to be reported in this paper, there is now available at Onderstepoort a complete series of accurately aged ovine foetuses. By direct comparison with this standard unknown foetuses are aged with a fair degree of accuracy.

The very nature of this standard, excellent as it is, militates against the extension of its sphere of usefulness beyond local limits. The primary object of this paper is to place this standard at the disposal of workers farther afield. It is hoped that this aim will be achieved by the publication of a partly descriptive and partly statistical study of those features of the collection which appear to be of importance in age determination.

As the development of the foetus is intimately connected with changes in the maternal genitalia, organs of lactation and endocrine glands, all these aspects of the reproductive process have received attention and will be considered in this paper. In some instances, it is felt, the numbers of observations are too limited to allow of definite conclusions. Nevertheless, the figures are given in the hope that, with appropriate additions by other investigators, eventually a large mass of data will be accumulated.

In connection with the literature it must be pointed out that every effort has been made to avoid what may be termed "second-hand" quotations. However, where it has been impossible to obtain the original article in this country, it is felt that recourse to such quotation is preferable to losing entirely a useful reference.

Acknowledgments.

It is with great pleasure that I acknowledge my indebtedness to those friends and colleagues who have assisted materially in the completion of this work.

The facilities for undertaking this study were provided by Dr. P. J. du Toit, Director of Veterinary Services, and Dr. C. Jackson, Professor of Anatomy. To the latter I am particularly indebted on account of his continued interest and his willingness at all times to assist with sound advice. Moreover, in the final preparation of this paper I was able to draw heavily upon his masterly knowledge of literary presentation.

Both to Dr. Quinlan, Sub-Director of Veterinary Services, and to his assistant, at Ermelo Mr. van Aswegen, B.V.Sc., I am grateful for their assistance, especially in having the ewes tested and served. The figures for the specific gravities of the foetal fluids were kindly supplied by Dr. J. G. Louw, Biochemist. But for the advice and

guidance of Dr. Laurence, Statistician, I would have experienced much difficulty in arriving at an accurate interpretation of the vast amount of data collected.

My thanks are due to Messrs. C. G. Walker and T. Meyer for the efficient manner in which they have dealt with the figures and plates, and to Mr. F. D. Horwell, technical assistant in the Section of Anatomy, for his valuable services throughout the course of the work.

Finally, it is a pleasure to record my appreciation of the assistance rendered by my wife, not only through her deep interest, at all times an inspiration, but also in typing the entire manuscript and assisting with the graphs and tables.

CHAPTER 2.—REVIEW OF LITERATURE.

A search of the literature has emphasised the meagreness of authoritative knowledge of prenatal growth of domesticated mammals. Much of the available knowledge is contained in works of which foetal development was not the primary consideration. In such cases the information, being merely incidental, received but scant attention. This literature is, to some extent, supplemented by studies on small laboratory mammals, in which the general principles of growth are apparently similar to those in larger mammals.

For the sake of clarity the literature will be treated under headings corresponding with those to be employed in the consideration of the experimental data.

(a) Genital Tract and Placenta.

1. *Vagina.*

Anatomists differ as to the nomenclature of that portion of the female genital tract situated posteriorly to the cervix. The English school refers to the portion between the cervix and the external urethral opening as the vagina. Everything posterior to this is called the vulva. In German works this latter part is further divided into the vestibulum vaginae, which constitutes the major portion, while the term vulva is restricted to the external opening of the urogenital sinus. In this work the German teaching will be followed, except for the fact that vestibulum and vagina will be taken together and will be referred to as " vagina ".

Sisson (1927) found that the vagina of the sheep has a length of between 10·5 and 11 cm. He states that the labiae of the vulva are thick and firm and that the ventral commissure is prominent.

From a consideration of the anatomical situation of the vagina it would appear that, after having served as a portal of entry for the sperm, this structure does not again participate in the reproductive process until the approach of parturition, when it dilates to allow of birth. On account of its relative unimportance this organ does not receive attention in embryological texts. However, it seems

obvious that during the period of pregnancy certain changes must occur and that the purport of these must be to render the vagina a larger and more easily distensible organ.

It has long been noted that with the approach of term there is an enlargement and a relaxation of the vulva. Craig (1912) and Williams (1917) refer to the soft and flabby nature of this organ prior to parturition. Hammond (1927) states that "just prior to parturition the vulva and the vagina become red and swollen." While studying parturition in the mare, Holzgruber (1925) observed that these changes are recognisable as long as three weeks prior to the birth of the foal.

Hammond (1927) finds that the vaginal pregnancy changes resemble closely those of the oestral cycle. They are mostly of a microscopic nature. In the anterior half of the vagina the changes are similar to those of the adjacent portion of the cervix. In this portion of the vagina is found a fair amount of sticky mucous material. Opperman (1922) states that during pregnancy the vaginal wall has a dry and sticky feel.

2. *Cervix.*

In the ruminant the cervix is an extremely well-developed structure, with walls that are thick, dense and inelastic (Ellenberger & Baum, 1921). The cervix of the sheep has a length of about 4 cm. and its lumen is practically obliterated by reciprocal prominences and depressions of the mucous membrane (Sisson, 1927). Trautmann (1917) also indicates that in this species the uterine seal is well-developed. The presence of such a seal during pregnancy is mentioned by many authors. For the cow, Williams (1917) gives details of the cranio-caudal development of the mucous plug. Woodman and Hammond (1925) show how in the bovine the actual quantity of mucous material increases steadily throughout pregnancy. There can be no doubt that this accumulation of mucus effectively closes the uterine canal during the major part of the gestation period.

In dealing with the physiology of parturition Craig (1912) states that there is a softening and a loosening of the cervical walls "a few days prior to parturition." Zeiger (1908) shows that there is also an enlargement of the cervical canal towards the end of pregnancy. In the bovine he finds that by the end of the seventh month the canal is sufficiently open to allow of the introduction of one finger. Hammond (1927) is of opinion that, apart from sealing off the uterus, the steady accumulation of mucus in the cervix assists in the dilation of this structure.

3. *Uterine Body and Horns.*

The uterus of the sheep is bicornuate. According to Sisson (1927) each horn has a length of from 10 to 12 cm. As a result of the gradual tapering of the anterior extremities of the cornua there is no definitely discernible external landmark indicative of the exact point of junction of the horn with the Fallopian tube. Posteriorly the two horns unite to form the body. The arrangement of the peritoneal covering makes this undivided portion appear longer than it is. The approximate length of the body is 2 cm.

Roux (1936) gives the weight of the uterus of the non-gravid Merino sheep as varying between 15·5 and 77·5 gm. He deals with eight groups of sheep, some of which, having been fed on low level rations, were in very poor condition. In these latter sheep the extremely low uterine weights were encountered. Although he does not find it possible to demonstrate a definite correlation between the weight of the ewe and the weight of its uterus, Roux nevertheless concludes that nutrition does exert an influence on uterine development. It is not possible to determine to what extent previous pregnancies might have affected the figures given by him.

Favilli (1928) gives the weight of the ovine uterus as varying between 40 gm. in virgin ewes, and 65 gm. in previously pregnant sheep. His observations, having been made in the slaughter-house, are not likely to represent poor conditioned sheep. This might explain the absence of figures as low as those mentioned by Roux. Favilli gives the weight of the uterus at term as 1,010 gm. This figure appears somewhat high and in a later publication (Favilli, 1929), it is stated that such a figure is obtained in cases of twin pregnancies. With a single foetus the figure is about 600 gm. Favilli (1928) quotes figures to show that similar increases in uterine weight occur in the bovine. Malan and Curson (1937) present figures for the weight of the empty uterus at the end of each month of pregnancy. These show that there is a steady increase in the weight of the organ from about 60 gm. up to about 700 gm.

Rorik (1907) shows that in the bovine there is a definite increase in the weight of the uterus during pregnancy. During the first half of pregnancy this increase is most marked. What few figures are given by Hilty (1908) seem to support this view. Hammond (1927) obtains his figures from heifers pregnant for the first time, thus eliminating the effects of previous gestations. These figures show that by the end of the period the non-gravid weight of the uterus has increased almost twentyfold.

Figures for the pig (Stegmann, 1923) show that by the end of the thirteenth week of pregnancy the non-gravid weight of the uterus has been doubled.

Draper (1920) gives a complete series of weights for the guinea-pig uterus throughout pregnancy. These figures indicate that the growth curve of the uterus is of double parabolic nature, i.e. first slow, then increasing rapidly and later slowing down again. This type of curve had already been suggested by the figures quoted above for the bovine and the sheep.

In all these cases it is the weight of the empty uterus that has been considered. This figure is the sum of the weights of the uterine wall and of the placenta. As will be seen later, the placenta undergoes a great deal of growth during pregnancy and it exhibits its own type of curve. It is evident, therefore, that the weight of the empty uterus is influenced greatly by the inclusion of the placenta. In the ruminant it is an easy matter to separate the placenta from the uterine wall and thus obtain the weight of the latter alone. However, no such figures appear to be available.

As might be expected, the increase in weight of the uterus is accompanied by an increase in size. For the sheep Favilli (1928) shows that the area of the uterus increases from less than 100 sq. cm. in the non-gravid state to almost 1,600 sq. cm. at the end of gestation. He also demonstrates (1929) that there is a steady daily increase of the volume of the uterus. Stegmann (1923) shows that in the pig the length of the uterine horn becomes increased during pregnancy.

Favilli (1928) maintains that the increase in size of the uterus is brought about not only by a multiplication of muscle fibres, but by the enlargement of existing fibres. He gives measurements in support of this view. Hammond (1927) also mentions this point and indicates that during pregnancy the uterine muscle appears sparsely nucleated—an indication of the enormous increase in the cyptoplasm of the cells.

Favilli (1929) draws attention to the decrease of the thickness of the uterine wall during pregnancy. From the fact that the greatest reduction occurs fairly early during gestation he concludes that passive stretching is not the sole cause of the thinning. He states that, due to the even distribution of pressure in the liquid contents of the uterus, the thinning is spread evenly throughout the uterine wall. In the goat the non-gravid thickness of 8 mm. is reduced to 1·5 mm. near term (Zeiger, 1908). This author maintains that the reduction is greater in the pregnant horn " where the pressure is higher." Hilty (1908) demonstrates similar reductions in the case of the bovine.

Further information regarding the effects of pregnancy upon the uterus may be obtained from comparisons between virgin and involuted uteri. For the sheep Behne (1929) finds that the effect of pregnancy is to increase enormously the blood and lymph vessels. Not only is there an increase in size, but also in number. The elastic coats of there vessels are greatly thickened. For the goat similar results are reported by Hackeschmidt (1920). The changes in the structure of the bovine uterus, observed by Kraft (1923), resemble closely those of the sheep (Behne). Kraft finds that the microscopic picture of the involuted uterus is so typical as to allow of immediate diagnosis of a previous pregnancy. Sommer (1912), also dealing with the bovine, makes the statement that after pregnancy the uterus never regains its virgin state. The weight of the uterus becomes permanently increased in the approximate ratio of 2 : 1. He maintains that as a result of previous pregnancy there may be an inequality in the size of the two uterine horns. In the pig Stegmann (1923) gives the weight ratio between virgin and involuted uteri as 1 : 2. Richter (1936) agrees that this is so when average figures are considered, but he maintains that in individual cases there is much overlapping. He finds that a definite diagnosis of previous pregnancy can be made from the enormous thickening of the *elastica interna* of the mucosal arteries, from the tortuous course of the uterine rami of the middle uterine artery and from the thickened, translucent appearance of the *ligamenta lata*.

A further important uterine change associated with pregnancy is the increase in the blood supply. The increase in the vascular bed of the uterus has already been mentioned. Barcroft and Rothschild

(1932) have determined the actual amounts of blood present in the rabbit uterus at different stages of gestation. In the resting state the organ contains about 2 c.cm. of blood. From the fifth day the uterus shows an increased vascularity and by mid-term the content has reached 10 c.cm. From now onwards the embryos begin to grow rapidly and the blood content of the uterus increases at a similar rate until the maximum of 30 c.cm. is reached at the 27th day. Just prior to parturition there is a rapid diminution in the blood content of the uterus. That this increase in vascular content is not due to simple stagnation of blood is shown by Barcroft, Herkel and Hill (1933). They prove that there is a corresponding increase in blood-flow to the uterus and that this process anticipates foetal growth. The increase in size and pulsation of the middle uterine artery, mentioned by Williams (1921), is well known as a means of diagnosing pregnancy in the cow. Hammond (1927) also mentions these points, although his observation was limited to one cow well advanced in pregnancy.

From the above it is evident that during pregnancy the weight of the uterus is greatly increased due to the presence of more muscular tissue, enlarged and multiplied vascular tissue and increased blood-content. In addition it is seen that in part these changes persist after parturition, and that any resting uterus can on morphological grounds be assigned to one of two groups—virgin or involuted. In comparing uteri the existence of these two classes must be borne in mind. Especially in the non-gravid and early pregnant stages will the differences be of importance. Later in pregnancy these class differences will tend to be obscured by the changes induced by the existing pregnancy.

Some workers have considered the weight of the gravid uterus. This is of interest in that it gives the weight of the entire foetal system. Knowledge of the growth in weight of this system is useful in the study of growth or fattening of pregnant animals—it allows of a correction being made for foetal material. In the bovine Bartlett (1926*b*) has studied this matter from a different angle and has constructed a table giving the weight of the cow at each stage of pregnancy relative to her farrow weight.

For the sheep Curson and Malan (1936) tabulate the weight of the gravid uterus from 3 to 149 days. The increase is from 163 gm. to 3,700 gm. It must be pointed out that the high value at 3 days is due to the authors having included under "gravid uterus" the vagina, vulva, tubes, ligaments and ovaries. In a further study (Malan and Curson, 1937) the weight at 30 days is found to be approximately 130 gm., while the weight near term is given as 5,320 and 6,058 gm. The authors express the weight of the gravid uterus as a percentage of the nett body weight (i.e. total body weight of ewe less the weight of the gravid uterus) of the ewe, and then these values are plotted against gestation age. The result is a parabolic curve for which a fairly simple formula is given.

Bergmann's (1922) figures indicate that in the bovine the weight of the gravid uterus increases fifty-fold between the second and the tenth months. The increase is more rapid in the later months, hence the data would fit a curve similar to that given for the sheep.

Bergmann makes the statement that from the weight of the gravid uterus, foetal age may be determined with as much accuracy as from the weight or the length of the foetus itself. According to Hammond's (1927) figures for the bovine the increase of the gravid uterus from the beginning of pregnancy till the approach of parturition is one hundred-fold.

Brodermann (1921), dealing with the sow, maintains that the weight of the gravid uterus is very variable. Contributing causes to this variability are previous pregnancies, the number of foetuses and the amount of fluid present.

For the guinea-pig Draper (1920) presents figures which indicate that the position here is approximately similar to that already described for the sheep.

4. *Placenta.*

The sheep has a cotyledonary placenta—*semiplacenta multiplex*—(Zeitschmann, 1923). The cotyledons are made up of two parts, maternal and foetal. In the sheep the maternal cotyledon is concave and encloses the foetal component. In the non-gravid uterus the cotyledons are exceedingly small and can be recognised merely as small circular elevations on the surface of the uterine mucosa. During oestrum they appear to be somewhat more prominent. Ellenberger and Baum (1921) state the total number of cotyledons in the sheep to be between 88 and 96, arranged in each horn in four longitudinal rows.

By Assheton (1905) the diameter of the ovine cotyledon at 60 days' pregnancy is given as about 2 cm., while at full term the figure lies between 2·5 and 3 cm. Natural-size illustrations of the sheep's cotyledon at mid-term given by Zeitschmann (1923) indicate that at this stage the diameter lies between 2 and 3 cm. It is evident that the small cotyledon of the non-gravid uterus must undergo enormous growth during pregnancy. These few figures suggest that the major portion of this growth occurs during the first half of gestation. In the bovine the growth of the placenta has received some attention. Rorik (1907) records the weight of the placenta at 7 weeks as 12 gm. and at 26 weeks as 5,200 gm. From his figures there does not seem to be any further increase after this stage. He shows how the ratio of foetal to placental weight widens with the advance of gestation. The area of the placental surface increases from 0·04 sq. metres at 7 weeks to 45·25 sq. metres at 31 weeks. Hilty (1908) traces the increase in size of the largest cotyledon. The diameter increases from 0·6 cm. in the virgin uterus to 7·8 cm. at the 26th week, after which the increase, if any, is only very slight. Similar results are reported for the increase in height of the cotyledon and in the length of its peduncle.

The idea of rapid enlargement of the placenta during the first part of pregnancy, followed by a period of little or no change, is supported by the figures for the rabbit given by Lochhead and Cramer (1908). Here placental weight is shown to increase rapidly up to the 22nd day, after which it remains stationary apart from a slight decrease at the approach of term. For the guinea-pig Draper (1920) supplies some data. Unfortunately foetal membranes and

placenta are grouped together, hence the use of the figures here is open to criticism. Nevertheless, Draper's graph shows a period of rapid increase followed by a period of little development.

In the bovine the number of functional cotyledons has been considered. Rorik (1907) reports wide variations in the total number of cotyledons—70 to 120. He shows that the number in the horn bearing the foetus is always in excess of that in the other horn, sometimes by as much as 150 per cent. He encountered accessory cotyledons in one-third of his cases. The presence of these was not necessarily associated with a diminished number of true cotyledons. Bergmann (1922) also reports a preponderance of functional cotyledons in the gravid horn. By counting the numbers of cotyledonary attachments he concludes that in the gravid horn no more attachments occur after the second month, whereas in the non-gravid horn these continue to be formed until the end of the third month.

The mode of attachment of foetal to maternal cotyledons has been studied by Assheton (1905). In the sheep the first attack upon the maternal uterine mucosa occurs between the 17th and 18th days *postcoitum*. At this stage the attachment is most frail, the membranes being held in position mainly by the pressure of the foetal fluids. At about the 30th day villi appear on the chorionic surface, resulting in a closer attachment. At the 44th day the foetal cotyledons are visible and have convex free surface which fit into the concavities of the maternal cotyledons. Assheton states that by the 78th day the general character of the fully formed placenta is apparent and that from this time the only change is a slight increase in size. He also mentions the presence of large reddish-black coagula in the centres of the fully formed cotyledons.

5. *Fallopian Tubes.*

In the sheep the uterine tube has a length of 14 to 16 cm. (Ellenberger and Baum, 1921). The gradual merging of the tube with the horn of the uterus has already been mentioned. Lee (1929) maintains that in most mammals there is a more-or-less well-developed valve at this junction. The sheep is not specifically mentioned, nor is it implied that the junction is always readily recognised from the exterior.

In none of the standard embryological or obstetrical textbooks is any mention made of specific pregnancy changes in the Fallopian tubes. Bergmann (1922) concludes that pregnancy has no influence on the length of the tubes. Lambert (1928) states that in the horse, the bovine, the cat and the dog he has found, associated with gestation, extensive vascularization of the tubes accompanied by hypertrophy of connective and muscular tissue and a distinct increase of elastic tissue.

(*b*) Foetal Membranes and Fluids.

1. *Membranes.*

The formation and the appearance of the foetal membranes of the ruminant are well described by Zeitschmann (1923). He shows that there are two sacs, the inner being the allanto-amnion and the

outer the allanto-chorion. Like Craig (1912), Zeitschmann states that early in pregnancy the outer sac increases rapidly and soon comes to fill the entire uterine cavity. Meanwhile the inner sac has remained relatively small, investing the foetus closely. Later on the amniotic sac expands rapidly and soon it occupies a large portion of the chorionic space, practically obliterating the central part of the sac. At this stage the concentric arrangement of the two sacs is not very evident and the appearance is more that of a large amnio-allanto-chorionic sac with two allanto-chorionic appendages—one at either pole. These latter two sacs retain their communication with each other and with the umbilical infundibulum. This relationship is well indicated in a sketch given by Bailey and Miller (1921).

The appearance of the ovine foetal sacs is described by Assheton (1905). He shows how at 14 days *post-coitum* the blastocyst has extended the full length of both uterine horns. At this stage the membrane is very frail, is transparent and absolutely colourless. Unfortunately he does not trace the further development of the membranes to show at what rate these delicate sacs grow into the relatively large structures which come away as the afterbirth of the ewe. Malan and Curson (1937) give figures for the weight of the membranes during pregnancy. These show that the total weight increases steadily throughout the period, although this increase is not shared equally by the two components. The allanto-chorion is shown to undergo little or no increase after the second month, while the allanto-amnion increases steadily for four months, after which the rate of increase declines.

The weights of the membranes at different stages of gestation in the bovine are recorded by Rorik (1907) and by Hammond (1927). From these sources it is seen that just prior to parturition the membranes have attained more than 1,500 times their weight at the end of the first month of pregnancy. In addition Hammond's figures show that the membranes grow at a steady rate throughout the period.

The fact that Draper (1920) has included the weight of the placenta in his figures for the membranes of the guinea-pig, renders these data rather unsatisfactory. However, if it is assumed that during the second half of pregnancy the increase in weight of the placenta becomes negligible, then Draper's graph would tend to indicate a steady growth of membranes throughout pregnancy. Hammond (1937) deals with the foetal placenta and membranes of the rabbit. His graph shows that there is considerable increase in these structures and that a fairly steady rate of growth is maintained throughout the period of gestation.

2. *Fluids.*

Needham (1931) gives a summary of most of the available knowledge on the foetal fluids. Most of the researches quoted by him are spasmodic investigations on isolated chemical constituents of the fluids. For the purpose of an inquiry into the origin of the foetal fluids Paton, Watson and Kerr (1907) selected the sheep as their subject " because, in this species, both fluids are present in relatively large quantities throughout the entire foetal period ". The figures

supplied by the authors bear out this statement. Moreover, they show that both fluids undergo changes in physical and chemical properties with the advance of gestation, while at the same stage of pregnancy the two fluids differ. Malan and Curson (1937) show that, in the sheep, the total amount of fluid increases steadily with advancing foetal age. They find that the allantoic fluid shows little increase during the first three months, to which period the increase in the amount of amniotic fluid is mainly confined. Zeitschmann (1923) gives the amount of fluid at parturition as between 50 and 450 cc. for the allantoic and between 150 and 400 cc. for the amniotic.

For the bovine Bergmann (1922) shows that the quantity of foetal fluid increases rapidly during the first half of pregnancy, while later this increase is much less. Hammond (1927) indicates that during the first half of pregnancy the increase in weight of the foetal system is due mainly to the accumulation of fluid, whereas towards the end of the term it is accounted for almost exclusively by foetal growth. Bergmann finds that what increase does occur after the fifth month concerns mainly the allantoic fluid. The latter fluid is present in greater amount than the amniotic. Zeitschmann (1923) asserts that the ratio at full term is 3:1 in favour of the amniotic fluid.

Hammond (1927) draws attention to the fact that cessation of increase of the foetal fluids and inception of secretory activity of the mammary gland both occur at about the fifth foetal month. He suggests that this may be significant, and no mere coincidence. In the case of the rabbit (Hammond, 1937) it is also seen that there is a steady increase in the amount of fluid up to the 24th day, after which there is absorption of fluid, so that at birth only a few drops remain. The absorption is active on the part of the foetus itself, but the decrease in amount tends to indicate a cessation of fluid formation. If this is so, then in this species too there is apparently some correlation between mammary secretory activity and inhibition of foetal fluid formation.

In the guinea-pig Draper finds that the amount of amniotic fluid when plotted against gestation time gives a double curve, indicating at first an increasing rate of formation and later a decrease in this rate. Until the middle of the period of gestation the amniotic fluid weight exceeds that of the foetus. Afterwards the foetal weight is the greater, and the ratio between it and the amniotic weight becomes wider with the advance of pregnancy.

(c) The Foetus.

1. *Foetal Age.*

Although MacDowell *et al* (1927) speak of foetal age as distinct from age of the zygote, most workers use the term as indicative of the total age of the organism, from the time of fertilization of the ovum until the moment of death or examination of the foetus (Arey, 1931). In most cases it can be assumed that the time of death of the foetus coincides with that of the expiry of the mother. Especially is this the case where the mother is perfectly healthy right up to the time of slaughter. In the human being, where

available knowledge is not sufficient to allow of accurate determination of the time of fertilization, such terms as "copulation age" and "menstrual age" have been introduced. Neither of these gives the true foetal age.

In order to be able to determine the exact time of fertilization it is essential to know the time of ovulation, the rate at which the sperm traverses the female genital tract and the vitality of both ovum and sperm in the uterine passages. As a result of the investigations of Quinlan and his co-workers (1931 and 1932) a detailed knowledge of all these factors in the Merino sheep is available. They have shown that ovulation occurs at about the 30th hour after the onset of oestrum, and that the unfertilized ovum soon loses its vitality. The male sex cell reaches the Fallopian tube, in which fertilization occurs, within six hours of copulation, while it retains its vitality for from 12 to about 36 hours. If, therefore, service has been allowed at such times during oestrum as to ensure a large number of active sperms being available in the tube at the time of ovulation, then it may be taken that fertilization occurs without delay i.e. at about the 30th hour after the onset of "heat".

The methods of determining the onset of oestrum and of calculating foetal age will be considered in the following chapter.

2. *Factors Affecting Growth of the Foetus.*

In dealing with foetal growth it is essential that due consideration be given those factors which may influence development of the foetus. Where possible these factors should be controlled. Failing this they should at least be borne in mind in the interpretation of results. On consulting the literature one finds that in nearly all cases birth-weight has been the criterion as to whether a factor has influenced foetal growth. Under these circumstances, therefore, even after the influence of a certain factor has been established, there is still the difficulty of not knowing whether this influence was exerted with equal force throughout the entire prenatal period or whether it had been intensified at certain stages.

The painstaking efforts of MacDowell and his collaborators to ensure pure strains and absolute accuracy in ageing have failed to eliminate considerable variation in the weights of foetuses of identical age. No doubt the causes of such variations are highly complex and only by the most extensive research could they be checked with accuracy. Even to attempt a detailed discussion of this problem is far beyond the scope of this work, hence but a few of the more obvious points will be mentioned.

Firstly, *material influences* may be considered:—

Breed and Strain.

To elaborate on the differences in size and conformation encountered in different breeds and strains of the same species, would be superfluous. It is shown by Fitch, McGilliard and Drumm (1924), that not only is there a difference in birth-weight in four milk breeds, but that the ratio of calf to maternal weight also differs. This

indicates that the birth-weight differences cannot be regarded merely as proportional increases or decreases according to maternal size. In a detailed study of two closely related strains of albino rats Freudenberg (1932) shows that apart from differences in body weight, there are present, even at birth, distinct differences in organ weight-ratio.

These few comments should suffice to demonstrate that in studies on prenatal growth it is essential that material be drawn not only from the same breed but also, as far as is possible, from the same strain.

Size and Condition.

His observations on both pure- and cross-bred sheep lead Russel (1919) to state that in nearly every case is size of the lamb determined by size of the ewe. Humphrey and Kleinhenz (1908) maintain that large ewes produce lambs heavier than those from small ewes. Hammond (1932) mentions a crossing experiment at the University College, North Wales. Welsh ewes were bred to Border-Leicester and to Southdown rams. Although the former rams are large animals, ewes served by them lambed with only very little more trouble than did those bred to the Southdowns. For the rat, King (1915) states that as the weight of the mother increases, so does the birth-weight of her young become greater. However, as in the rat weight and age are correlated, it is possible that the greater maturity of the heavier females played some part in her investigations.

Hammond (1932) points out that the maternal influence over foetal development is twofold—genetic and nutritional. He shows that in a favourable season, during which the ewes were in good condition prior to lambing, the birth-weights were high. Further, when ewes were divided according to condition into good, medium and poor groups, it was found that the average birth-weights followed the same order. The difference between the first two groups was much less than that between the medium and the poor groups. It appears that improvement in condition beyond a certain stage is not reflected in the birth-weights of the offspring from such ewes. In fact it is found by Quinlan and Roux (1936) that extremely fat cows produce very light calves. This finding is in accordance with the results of Eckles (1919 and 1920). The latter concludes that, in the cow, nutrition of the mother has but little influence on the size of the calf at birth. Donald and McLean (1935) state that " increase in birth-weight as the lambing season advances is due to improvement in the condition of the ewes ". Apparently they contend that an improvement in the condition of the mother is rapidly communicated to the foetus. Hammond (1932) stresses the necessity for providing pregnant ewes with good feeding for six weeks prior to lambing. He may, however, be more concerned with procuring a good milk-supply subsequent to parturition than with actually producing a heavier lamb at birth.

Eckles (1919 and 20) and Fitch *et al* (1924) maintain that cows in quite poor condition produce calves of average weight. The latter authors show that only when nutrition is severely depressed is calf

weight affected. Even then the effect is not proportional to the loss of condition of the mother. This is demonstrated by the decrease in the calf-mother ratio. In their work on the breeding of cattle on pastures deficient in mineral content, du Toit and Bischop (1929) show that, between their "bone-meal" and their "control" herds, in spite of marked differences in condition, vigour and resistance to disease, there is no significant difference in birth-weight. Groenewald (1935) also fails to find a reduction of birth-size in calves born of mothers fed on rations deficient in various mineral substances. Barry (1920) maintains that severe inanition in pregnant rats causes a decrease in body weight of the resulting litter. The different organs of the foetus are not all affected to the same extent, hence a change in the weight-ratio of the organs results. Quinlan and Roux (1936) find that in cows subject to severe restriction of exercise and sunlight there is no change in either size or degree of development of calves, provided that this treatment does not result in excessive deposition of fat, when low birth-weights will be encountered.

Age.

According to age, breeding stock may be divided into three classes—immature, mature and aged. In practical animal husbandry the breeding ability and production of animals in each of these classes have received much attention. All this work is of great interest here, for it brings out one essential fact—that both as a productive and as a reproductive machine the mature female exhibits an efficiency far superior to that of the female in either of the other two classes.

Hammond (1932) refers to the inability of shearlings to produce lambs with a birth-weight equal to that of the offspring of mature ewes. Donald and McLean (1935) conclude that the influence of age of the ewe is sufficiently strong to obscure the effects of sex differences in the lambs.

Fitch *et al* (1924) find that mature cows produce calves larger than those immature cows. Eckles (1919-20) states that first calves are smaller than later ones, and that the maximum weights occur at the third to the sixth calving. Later in life, in the aged group, there is a decrease in birth-weight. It is well known that milk production increases in the second and third lactations and declines in aged cows.

King (1915) states that birth-weight in the rat increases with the age of the mother. Donaldson (1924) mentions that the albino rat usually produces its largest litters at the second to the eight gestations. Long (1906) states that the average weight per pigling in the case of year-old sows is about 800 gm., and that this increases with age until at 5 years the figure is 1,300 gm. Carmichael and Rice (1920) show that, in addition to this increase in the size of the individual pigling, there is also an increase in the number per litter as the age of the sow increases. These changes are evident up to the 4th litter. Schneider (1936), studying German pigs, reports similar results.

A further indication of the greater reproductive ability of the more mature female is the fact that in sheep multiple births occur with the greatest frequency in the most mature age-groups (Jones and Rouse, 1920).

In the foregoing reference has continually been made to the number of gestations as an index of maturity. In most cases this will also be indicative of age. But that maturity is actually a matter of age, and is not dependent on previous breeding, is shown by Joubert (1936). He finds that virgin ewes of mature age will produce lambs equal in weight to those of ewes of the same age which had previously been bred. Donaldson (1924) shows that if the female rat is prevented from breeding until she is four months of age, then the first litter will be as large as any of the subsequent ones.

To sum up the various maternal influences it may be stated that breed, strain and maturity are of importance in foetal development, whereas the state of nutrition, unless grossly beyond normal limits, does not exert much influence.

Paternal Influences.

It is quite evident that the influence of the ram on the foetal size is limited to the genetic sphere. Mumford (1901) declares that the sire does not exert any influence on the size of its offspring. Humphrey and Kleinhenz (1908) find that paternal influence is rather limited. Their method of investigation, however, was not calculated to allow for differences in age and breed.

Hammond (1932) cannot agree that the ram has no influence on the size of its lambs. He draws attention to the fact that cross-bred lambs often differ in size from the pure breed. This difference must be induced by the sire.

In dairy cattle Fitch *et al* (1924) find that the sire does exert an influence upon calf-weight but that the degree of such influence is somewhat limited. Eckles (1919-20) shows that the calf produced by a Jersey cow mated to a Fries bull is much heavier at birth than is a pure-bred Jersey calf. Quinlan and Roux (1936) also find that the bull has an influence on calf-size.

Additional factors which should be considered are:—

Number of Foetuses.

It has been shown by Murray (1921) and by Hammond (1932) that each member of a set of twins is lighter than a single foetus of similar age. The total weight of the set, however, exceeds that of the single individual. Between twins and triplets similar differences exist. These results are confirmed by numerous other workers. Schneider (1936) points out that in pigs the size of the individual pigling at birth decreases with the increase of the number per litter. King (1915) says that the same applies in the albino rat.

Zuntz (1909) shows that when rats are kept on unbalanced rations, the frequency of gestation and the number per litter are reduced much more than is the size of each individual foetus. Evidently, where nutriment is limited, reduction in number safeguards the size of the young.

Sex of the Foetus.

That the birth-weight of the male exceeds that of the female has been established for the human being (Jackson, 1909), the sheep (Hammond, 1932; Murray, 1921; Donald and McLean, 1935), the bovine (Fitch *et al*, 1924), the rat (King, 1915; Donaldson, 1924; Freudenberg 1932) and the pig (Schneider, 1936).

This difference in weight is not merely the result of an enlargement in all dimensions in the male. For the bovine it has been demonstrated by Keller (1920) and by Beer (1925) that somatic differences dependent upon sex do exist. These are detectable as early as the second month of prenatal life. The male is much more plump and is better muscled, especially in the cervical and the shoulder regions. The female is more slender and has a long, narrow head. The results of Keller were well controlled in that he used sets of twins of like and of unlike sex. Although variations were found in both groups he concluded that there was evidence of a definite inherent somatic sex-difference.

For the pig similar results are reported by Smestisko (1925). Jackson (1909) refers to sex-differences in human foetuses, and Scammon (1922) shows that in the height-weight index of new-born babies this difference is also discernible.

In addition to the above influences there is still to be considered the effect of the time of the year at which birth occurs.

Seasonal Influences.

Hammond (1932) and Donald and McLean (1935) find that birth-weight increases with advance of the lambing season. Apparently, however, they consider that this is the reflection of an improvement in the condition of the ewes. Carmichael and Rice (1920) find no regular birth-weight differences due to season, although they do state that piglings farrowed outside the regular seasons have smaller weights than usual. Schneider (1936) finds that the birth-weight of piglings farrowed in winter exceeds by 70 gm. per foetus that of similar young born in summer.

3. *Growth in Weight.*

Colin (1888) appears to have been the first investigator to study the growth in weight of the ovine foetus. A big gap in his figures between the ages of 57 and 120 days makes it impossible to determine the shape of the growth curve. Due to the absence of age figures the series of foetal weights given by Paton, Watson and Kerr (1905) is also useless for this purpose. These weights indicate only the range to be expected. Craig (1912) mentions 4 Kg. as the weight of the lamb at birth.

Curson and Malan (1935) give detailed figures for a whole series of Merino foetuses. In general their figures are lower than those of Colin. This may be due to their having worked upon a different breed. They show by means of a chart that when weight is plotted against age the result is a logarithmic curve. The latter

shows much fluctuation, especially after the age of 100 days. The authors state that "these fluctuations are well understood, for the series of lambs was taken at random, and there is considerable variation in the weight of individuals of the same age". In a subsequent paper [Malan and Curson, 1936 (*a*)] additional weights are recorded, and although these are not plotted against age, it appears that the curve so obtained would follow a course similar to that of the curve given in the earlier article. Further weights are given by Malan and Curson (1937), and these only serve to strengthen the previous findings of these authors.

For the bovine foetus a fair amount of data are available. Rorik (1907) and Bergman (1922) tabulate a considerable number of weights for foetuses of various ages. Craig gives the average weight of the calf at birth as 32·5 Kg. Hammond (1927) mentions the weights of eight accurately aged foetuses. In spite of many overlappings, and the fact that breed differences must be considerable, these figures all indicate that the age-weight curve for the bovine foetus is similar to that described by Curson and Malan for the sheep.

The same type of curve has been established for other mammals, e.g. the human being (Jackson, 1909), the guinea-pig (Draper, 1920), the rat (Stotsenberg, 1915) and the mouse (MacDowell *et al*, 1927). Apparently the same type of curve holds good for other mammals, and it appears possible to express all foetal growth-rates by the same general formula, the constants of which have merely to be adjusted for each species.

So far only absolute growth has come under consideration. When relative or percentage growth curves are considered there is a remarkable lack of unanimity amongst workers. When it is noticed that Brody [1927 (*a*)] using the identical data employed by Minot (1908), obtains radically different rates, then it is realised that the real problem here is of mathematical nature.

Brody [1927 (*b*)] presents graphs with which he attempts to show that the relative growth curve is not of a steadily decreasing nature, but that it is made up of a series of straight segments. MacDowell *et al* (1927) do not agree with this. They demonstrate an even and regularly decreasing curve. They criticise the data upon which Brody relied for his determinations, and they also show that any curve may be approximated by a number of straight lines.

4. *Growth in Length.*

In the introduction to this paper was mentioned the failure of investigators to define with accuracy their procedure. Nowhere is this failure more apparent than in the case of length of foetuses. By determining increase in length an idea of skeletal growth, rather than of mass increase, is obtained. Later it will be seen that these two factors are actually correlated. It is evident that in each case the identical dimension should be measured. This can be done only when the extremities of the line are clearly defined. If the line

is not straight then its course between the two fixed points should be indicated. Unfortunately in the literature the term " length of foetus " is often given without further amplification.

For the human foetus certain fixed measurements are in general use. These are described by Mall (1910). Commonest of these are sitting-height (crown-rump), standing-height (crown-heel), and vertebral column length. In the veterinary field most workers have, no doubt, made use of a dimension akin to the human sitting-height or crown-rump measurement. That the fixed points have varied is indicated by the few instances in which an attempt at defining " length " has been made. Hammond (1927) refers to a poll-base-of-tail measurement, while Curson and Malan (1935) use the forehead and the base of the tail as landmarks. For rats the nose-anus dimension is in general use (Donaldson, 1924). Bergmann (1922) refers to a muzzle-rump measurement employed by some investigators. He concludes that this is inferior to the poll-rump measurement.

In all these cases attempts have been made to indicate the length of the foetal body. All the measurements are of straight-line dimensions. Although " crown-rump " appears to be a most useful measurement in the human being, its value in an animal with a relatively long and flexible neck is greatly reduced by the possibility of large errors due to changes in posture. In the sheep foetus, even when it is laid out flat with the neck perfectly straight, relatively large variations may be introduced simply by flexing and extending the head on the neck. In making use of such dimensions it is essential that the position of the foetus as well as the landmarks be standardised and defined with care.

Malan and Curson [1936 (*a*)] express the opinion that a measurement along the back of the foetus, in preference to a straight line, should assist in eliminating the effect of posture. It was their intention to make use of Mall's (1910) " length of vertebral column " dimension. They employed as an anterior landmark for their " curved crown-rump " length, the midpoint of the line joining the medial canthi of the eyes. The posterior landmark was the root of the tail (Curson, 1937). The authors do not find that the use of this measurement improves the fit of the data in a weight-length curve. From this it would appear that the curved line is no more accurate than a straight measurement. The line used is obviously not the equivalent of that referred to by Mall, and it remains to be seen whether Mall's line—length of the vertebral column—would produce better results. The difficulty in making this measurement is that there is nothing to indicate that, in any species other than the human being, the extension of the eye-ear line indicates the point of origin of the vertebral column.

Lengths of sheep foetuses from the third to the twenty-second week are given by Gurlt (1847). His figures show no close similarity to those of other workers. Better figures are those of Assheton (1905). These are limited to the very young stages. A large amount of data concerning accurately aged Merino foetuses are presented by Curson and Malan (1935). On plotting these data against age the authors obtain a curve which resembles an elongated " S ". This

curve differs from the weight-age curve previously described. Due to the small number of foetuses used the data show much variability, but this cannot obscure the fact that there is a definite length-age curve for foetal growth. Apart from additional figures for straight crown-rump length, Malan and Curson [1936 (*a*)] also tabulate their "curved crown-rump" measurements. Although the latter figures are not plotted, it appears that this would result in a curve of roughly the same shape as that obtained with the straight-line dimension. Galpin (1935) indicates the lengths of a number of accurately aged Romney foetuses. The measurement taken appears to have been somewhat akin to Malan and Curson's [1936 (*a*)] curved crown-rump dimension. If the figures represent straight crown-rump lengths then a Romney foetus must be considerably larger than a Merino foetus of identical age.

Craig (1912), who simply speaks of "length of the foetus", gives figures for the horse, the bovine, the sheep, the pig and the dog. As he mentions merely the average length for each of the seven periods into which he divides the gestation period, no conclusions as to the nature of the growth curve may be drawn. His data for the sheep fit in well with the figures recorded by Curson and Malan. For the length of the lamb at birth Craig gives 18 inches, which is considerably higher than the figure mentioned for a 145 day foetus by Curson and Malan (1935). If Craig's figure is a good average one then the distinct flattening of the last part of Curson and Malan's curve should disappear and the curve should be much more even. This would then produce a curve resembling that given by Draper (1920) in his work on the guinea-pig. His curve also has a double nature, but the second change of direction is very gradual. Draper concludes that from the 15th day of foetal life till the 64th day the percentage rate of growth in length decreases steadily. Hammond (1927) indicates the measurements of eight bovine foetuses, ranging in age from 1 to 8 months. The figures would apparently fit a curve such as that under discussion.

Already it has been shown that there is a correlation between the age and the length of a foetus. In the human foetus this relationship has received a fair amount of attention. Simple empirical formulae for expressing each of these characteristics in terms of the other are given by Scammon and Calkins (1923). Noback (1922) demonstrates the simple relationship between sitting-height and standing-height. Scammon (1937) shows that the simple relationship between these two lineal dimensions may be extended to embrace numerous other external measurements of the foetus. Noback (1925) demonstrates that in the lineal growth of a single system such as the respiratory the age-length correlation is still present.

Curson and Malan (1935) consider the correlation between weight and length. In addition to presenting a graph for these two characteristics plotted one against the other, they show that relative increases in length and in weight may be plotted to a straight line. This is confirmed in a later article [Malan and Curson, 1936 (*a*)]. It is also found that the "fit" is not improved by the use of the

curved crown-rump measurement. They conclude that increase in length is a function of body weight, and that the reverse also holds good. They observe that the logarithmic values of weight and of crown-rump length are equally variable. Hammond (1927) states that "weight is more variable than length". Curson and Malan (1935) show that when Arey's (1934) data for the human foetus are plotted, the same length-weight relationship becomes evident. According to Scammon (1922) there is a definite height-weight index for babies at birth.

Workers on post-natal growth have not been content with measuring one dimension. Instead they make use of a number of representative measurements, some of which are mentioned and illustrated by Ragsdale, Elting and Brody (1926). It will be seen that there are two classes—straight and curved or circumferential. The purpose of all this is to obtain an idea of growth in all three dimensions, rather than to be limited to a "one-dimensional" conception of the process. It appears that by an extension of this idea to the study of foetal growth a much clearer and more accurate visualization of the changing proportions of the developing foetus may be attained. It is conceivable that ratios between dimensions may prove to be better indices of age than the absolute measurements themselves.

5. *Growth in Surface Area.*

Not only weight and linear dimensions but also surface area may be used as a measure of size. In the living animal, especially after birth, surface area assumes an even greater importance, for it is intimately associated with heat regulation and metabolism. In the sheep foetus this aspect of growth has been studied by Malan and Curson [1936 (*b*)]. They find that the curve for surface area against age is similar to that for weight and age. Relative increases in area and in weight may be plotted to a straight line.

Owing to the desirability of preserving intact the foetuses used in the present work, surface area has not been considered.

6. *Changes in External Appearance.*

It is not intended to discuss here the earliest developmental stages of the sheep embryo and foetus. Rather is it the object to commence at the stage at which in textbooks the subject is usually left, and to consider the manner in which the early common mammalian type is moulded into the Merino lamb delivered at parturition. The changes which take place will be correlated with age so as to arrive at something similar to Arey's (1934) "Table of Correlated Human Development".

Assheton (1905) intimates that the development of the sheep up till the 10th day has been worked out by him (1903), and that the further development has been fully described by Bonnet (1895 and 1898). Unfortunately neither of these papers was available to me.

For the bovine Hammond (1927) presents a table which, in spite of the small number of foetuses available for its compilation, is a useful guide in age-determination. An interesting feature of this

table is the use that is made of the stage of pigmentation of the eye in the early foetuses. Attention is also directed to the value of the limb-bud and the development of the hoofs in ageing foetuses. Some further details concerning the external form of bovine foetuses in the early stages of gestation are mentioned by Kupfer (1936).

The appearance and distribution of hair on the foetus has long been used as a criterion of age. The spread of hair over the Merino foetus is described by Duerden and Ritchie (1924). The time of the first appearance of hair is placed towards the end of the 2nd month. To this exception is taken by Curson and Malan (1935), who maintain that hair is first visible on the forty-second day of prenatal life. Galpin (1935) describes the development of hair in the New Zealand Romney foetus. She draws a distinction between tactile hairs and body hairs. The former start to appear on about the 76th day, whereas the latter are first seen on the 90th day. These times refer to the actual emergence of the hairs above the surface of the epidermis. The hair follicles, of course, are distinguishable long before this. Like Duerden and Ritchie, she finds that the development of hair is furthest advanced on the most anterior parts of the body. The backward spread takes place in accordance with a plan so regular that it appears to offer an excellent means of age-determination.

Craig (1912), Bergmann (1922) and Hammond (1927) give details for hair development in the bovine. When due allowance is made for the increased length of the developmental period in this species, it is seen that the figures mentioned correspond closely to those obtained for the sheep.

Other external features do not appear to have received much attention.

(d) The Maternal Mammary Gland.

Probably on account of the relative unimportance of the sheep as a milk producer, its udder does not appear to have excited scientific interest. That milk production in the sheep is of economic importance, especially in crossing for fat lamb production, has been shown by Joubert (1936). From the above it must not be assumed that in other species the causes of mammary development and milk secretion have not been the subject of scientific investigation. On the contrary, much work has been done in this connection.

Early in the present century Lane-Claypon and Starling (1907) studied with inconclusive results the effects on mammary development of various foetal placental extracts. Loeb and Hesselmann [1917 (a) and (b)] show that mammary activity is correlated with the oestral cycle as well as with pregnancy. The changes occurring with each oestral cycle resemble closely those of the early part of gestation.

A close study of the whole problem has been made by Turner. This author (1930) traces the foetal development of the udder, indicating the stage attained at birth. In a later paper (1934) development in the post-natal period is followed up and it is shown that, excepting for the deposition of fat, there is no change between

birth and the onset of puberty. From this time onwards, with each recurring oestral cycle, there is more and more growth and branching of the duct system and the gland tissue comes to resemble a tree without leaves. With the onset of pregnancy there is still more branching activity, followed by alveolar development. At mid-term the leafless tree picture has been altered to resemble a bunch of grapes, the ducts being the stalks and the alveoli the grapes. Now secretion commences and it is the accumulation of the secretory product which results in the visible filling out of the mammary gland. Hammond's (1927) results are in agreement with these views. Woodman and Hammond (1923) and Asdell (1925) find that the watery secretion which, in virgin heifers, may be withdrawn from the udder, begins at mid-term to undergo qualitative and quantitative changes which foreshadow the formation of the cholostral milk. The above results appear to explain why workers such as Ragsdale *et al* (1924) have found that in lactating cows a subsequent pregnancy does not have any effect upon milk yield until the end of the fifth month of gestation.

At the time of fertilization, therefore, mammary development is already under way. The further development of the udder occurs in a slow and orderly manner and is spread out over the entire period of gestation. It is incorrect to regard milk secretion as being initiated rapidly a short while prior to parturition.

This internal development and the accumulation of fluid must make itself evident in an increase in size and weight of the gland. Hammond (1927) shows that in heifers in their initial gestation period, the weight of the udder increases from 2,200 gm. at the end of the first month to about 3,000 gm. at mid-term, and to between 6,000 and 9,000 gm. shortly before parturition. Thus the major portion of the increase in weight occurs in the second half of pregnancy. Hammond further indicates that in considering udder weights, especally in dry cows, the age and the condition of the subject should receive due attention, as both these factors have an influence on the weight of the organ.

(c) The Maternal Endocrine Glands.

1. *The Pituitary.*

This gland is most intimately associated with all the sexual functions of the animal body. A sex-difference in pituitary size has been demonstrated in man (Rasmussen and Herrick, 1922), in the albino rat (Jackson, 1913; Hatai, 1913; and Addison and Adams, 1926) and in the pigeon and the dove (Riddle and Nussmann, 1933). In each case it is the female that has the larger pituitary, and in this sex the gland also has a steeper growth curve. Addison and Adams show that 95 *per cent* of the excess weight is accounted for by the anterior lobe of the hypophysis while the remaining portions are but slightly heavier than in the male. This is in agreement with the conclusions drawn by Rasmussen and Herrick from a study of a few human pituitaries. Working with albino rats, Andersen (1933) shows that at different times in the sexual cycle the hypophyseal weight varies. The maximum weight is encountered during oestrum. From then onwards there is a gradual decrease to the minimum

weight which is reached twenty-four hours prior to onset of the next heat period. In the fowl it has been shown that during the laying period the pituitary is heavier than during the moulting stage (Marza and Blinov, 1936). For the mare Schopf (1935) gives pituitary weights at different stages of gestation. He states that due to differences in the size of his subjects, no conclusions may be drawn from the absolute weights. When relative weight is the criterion then it appears that pregnancy is accompanied by an increase in pituitary weight. Whether Schopf is justified in assuming that pituitary size is proportional to body weight is questionable. Upon this point no direct evidence is available, yet it must be admitted that in the results of Addison and Adams (1926) some correlation between body weight and pituitary weight is evident.

From a study on the albino rat, Herring (1920) concludes that the effect of pregnancy is to reduce the weight of the pituitary. Hammond (1927) considers the oestral cycle to be the homologue of the reproductive cycle with dioestrum corresponding to the gestatory period. If this is the case, Herring's view receives confirmation from Andersen's (1933) findings.

In the bitch (Wolf, Cleveland and Campbell, 1933), in the sow (Cleveland and Wolf, 1933), in the mare (Schopf, 1935) and in the sheep (Warbritton and MacKenzie, 1937) it has been shown that widely differing histological pictures are associated with the various phases of sexual activity. The changes take the form of qualitive as well as quantitive variations in the chromophil cells of the anterior lobe. The changes described in the bitch and the sow suggest that during pregnancy a decrease in the size of this lobe is to be anticipated.

2. *The Adrenal.*

In the albino rat the sex differences and the growth curves of the adrenals resemble closely those of the pituitary (Jackson, 1913; Hatai, 1913). Barker (1937) finds that the adrenal of the dog is lighter than that of the bitch. Mature females have heavier adrenals than immature bitches. In mice Deanesley (1928) demonstrates histological differences between male and female adrenals.

Andersen and Kennedy [1933 (*b*)] show that in female albino rats adrenal weight is very variable, and that factors such as age, body weight, sexual season and the presence of infection must receive attention. They contend that bacterial infections of the lungs make for increased adrenal weight. Motow (1937) does not agree with this. Rosenbluth and Gayet (1932) conclude that the adrenal is relatively stable, both in weight and in adrenin content, to changes in diet.

Andersen and Kennedy (1932) show that during oestrum the rat adrenal is heavier than in dioestrum. This increase during oestrum is associated with definite histological changes, the cortex being increased in thickness at this stage of the cycle. Nahm and McKenzie (1937) demonstrate variations in the adrenal of the ewe. .

Deanesley (1928) finds that in the mouse there is associated with pregnancy a degeneration of the inner portion of the cortex, described by her as the " inner dark staining zone ".

Herring (1920) maintains that during pregnancy the adrenals of the rat are slightly hypertrophied. This finding is criticised by Andersen and Kennedy [1933 (*a*)], who maintain that the figures do not show any significant changes. The latter authors, after reviewing all the available literature, conclude that the case for pregnancy hypertrophy of the adrenal has not been establised. They admit that there is a possibility that all species may not react in an identical manner. After careful consideration of all the available data they maintain that in the case of the human being, the rat, the mouse, the rabbit and the cat, the balance of evidence is against such a hypertrophy. Their own results, in which relative adrenal weight is employed, indicate that during pregnancy the adrenals resemble those of the dioestrus period, which again are lighter than the adrenals of the oestrus period. During lactation a significant rise in adrenal weight is evident.

Mutow (1937) finds the adrenals to be heavier at oestrus than during dioestrus. When he considers a combined group of pregnant and lactating rats, a weight slightly above the dioestral standard is obtained. This increase may be accounted for entirely by the lactating rats in the group.

3. *The Thyroid.*

Andersen (1933) states that the great variability in weight of this organ is due partly to the difficulty of dissecting it free from the surrounding tissue. Jackson (1913) shows that during growth the relative weight of the thyroid changes, being highest at birth. Zimmermann (1933) states that with increasing age the thyroid undergoes regressive changes, the isthmus in particular being affected. Orywall (1933) finds that there is a definite weight difference between thyroids from female rats at Leipzig and from similar rats in Stuttgart. This may be a strain difference or it may be of dietary origin.

With all these possible sources of variation, only exceedingly large changes in the size of the thyroid would be of significance. It is not surprising to find that Andersen (1935) concludes that she is unable to demonstrate changes due to sexual season.

Guggisberg (1933) maintains that in the pregnant woman the thyroid is enlarged to such an extent as to render the increase clinically detectable. He states that although there is hypersecretion from the thyroid, this cannot be termed hyperthyroidism because the thyroxin content of the maternal blood is not raised. The surplus secretion is for the use of the developing foetus, which, until late in foetal life, is devoid of its own supply.

Herring (1920) claims to have shown that in the rat there is a decrease in thyroid size during pregnancy.

4. *Corpus Luteum.*

It is now agreed that this body should be regarded as a temporary endocrine gland. Its function has been studied in great detail. With this aspect the present paper is not concerned. Only its macroscopic appearance will be considered here. The appearance of the ovine

corpus luteum, both in the oestral cycle and in pregnancy, has been well described and illustrated by Quinlan and Mare (1931). The microscopic appearances have been reported upon by Marais (1936). From these works it is learned that the corpus luteum of the sheep maintains its structure for a long time. The corpus luteum of menstruation shows its first signs of deterioration shortly prior to the next oestrum, while that of pregnancy remains unchanged until the approach of parturition. The latter corpus luteum tends to sink deep into the ovarian tissue, especially during the latter half of gestation. This fact may be responsible for the statements of certain observers to the effect that the size of the corpus becomes reduced during the second half of pregnancy.

From actual weights of bovine corpora lutea, Bergmann (1922) concludes that there is no decrease in size throughout the period of pregnancy. Kaltner (1923) shows that during the entire term the bovine corpus luteum conforms closely to the average weight of 4·26 gm. Even at parturition he could not detect macroscopic changes in the size and appearance of this structure. In twin pregnancies he finds that each of the two corpora is smaller than the average referred to above. He maintains that the presence of dead, mummified or macerated foetuses does not prevent degeneration of the corpus nor even the approach of a new ovulation. Hammond (1927) also finds that there is no change in the size of the corpus luteum during the course of pregnancy. He mentions that during gestation follicular atrophy is much more severe than during the sexual cycle. This tends to cause a decrease of ovarian weight, especially in the case of the ovary not containing the corpus luteum.

5. *The Pineal.*

No reference to the macroscopic appearance of this organ during pregnancy has been encountered.

(*f*) General.

1. *The Situation of the Foetus.*

From a consideration of the mode of liberation of the ovum and of fertilization it will be evident that the foetus should in most cases be situated on the same side of the body as the corpus luteum. From the figures of Kupfer (1923) and those of Curson (1934) it is seen that this is the case. In a small number of cases, however, "migration" occurs. This may take place via the abdominal route (Leopold, 1888) or it may occur by the internal or uterine route (Warwick, 1926). This latter type of migration is not possible in mammals which have a uterus duplex—e.g. the rabbit.

When more than one foetus is carried, the corpora lutea may be in one or in both ovaries. Irrespective of the distribution of the corpora between the ovaries, the foetuses are usually spaced evenly along the entire tract (Warwick, 1926). Even in a case of uniovular twins, Henning (1937) found the foetuses lying one in each horn.

In cattle it has been found that the foetus is carried in the right horn more often than in the left (Keefer, 1937). Clark (1936) shows that this is not due to more frequent ovulation from the right ovary. It appears, therefore, that in the bovine migration usually occurs from the left to the right horn. Clark failed to find justification for the belief that there is usually a relationship between the sex of the calf and the horn in which it is carried.

In the sheep, due to the prolonged persistence of the corpus luteum, it is a simple matter to determine whether ovulation has occurred from alternate ovaries or not. Quinlan and Mare (1931) find that usually there is alternation between the two ovaries, but that on occasion ovulation may occur twice or even three times in succession from the same ovary. Ruhl (1925) mentions the fact that in the human being after unilateral ovariotomy the length of the sexual cycle is not doubled. This, he states, indicates that there is no inherent mechanism preventing successive ovulations from the same ovary.

2. *Effect of Gestation on Maternal Weight.*

In view of the scarcity of data on prenatal growth, some investigators (e.g. Ragsdale, Elting and Brody, 1926) have attempted to gauge foetal development by noting the increases in weight of pregnant cows. This method at best can but give an indication of the growth of the whole foetal system, which is neither equal nor proportional to foetal growth.

Bartlett [1926 (*b*)] states that in experimental work on pregnant cows it is often necessary to be able to apply a correction for foetal weight. He is of opinion that the only increase in weight of mature pregnant cows is that due to the foetal system, while in immature cows there is actual growth up to the fourth month of gestation. The former class will regain its service weight after parturition, while the farrow weight of the immature cow is the same as that shown at the fourth month of pregnancy.

Reference has already been made to Curson and Malan's (1936) treatment of the weight of the gravid uterus as a percentage of the nett live weight of the ewe.

CHAPTER 3.—PLAN OF INVESTIGATION.

(*a*) Material.

Apart from a few references to some of the material already reported upon by Curzon and Malan, the data for this paper are derived entirely from observations upon a series of ewes slaughtered at Onderstepoort during the period April to November, 1937. These sheep, typical Merinos, were drafted from the available flock on the research station at Ermelo, Transvaal. The methods of sheep husbandry practised on this farm are described by Hoffman (1935) and Roux and Hoffman (1935). Information regarding the physiography of the station and its effect on Merino sheep is given by Roux (1936).

The known sexual history of the ewes has been arranged in tabular form (Appendix—A). As the ewes had not been kept under constant supervision from the time of attaining sexual maturity, it is certain that many occurrences of oestrum are not recorded. However, the information concerning previous services and pregnancies is complete and accurate.

Regarding the service of the ewes, the " controlled " method (Roux and Hoffman, 1935) was practised. " Teasing " was carried out twice daily and service was allowed soon after the detection of oestrum, and again twelve hours later. Where an ewe was still willing to stand for the ram at the next testing (i.e. 24 hours after the onset of oestrum) a third service was allowed.

Four rams were employed to serve the ewes. All were typical specimens of the Merino breed, of mature age (about six years) and of fairly uniform size (around 140 lb. live weight). The rams were used at random, no attempt being made to mate a certain ram to any particular ewe, nor were the two consecutive services of any ewe necessarily given by the same ram.

Most of the ewes were tested daily for 22 days following service, and where oestrum reappeared it was assumed that the ewe had failed to conceive. In the case of those ewes required for the study of the earliest stages of pregnancy this testing was impracticable. When slaughtered, many of these ewes proved to be non-pregnant.

Soon after completion of the testing (or, in cases where no testing was carried out, soon after service) the ewes were railed to Onderstepoort, where they were placed in a small camp (100 x 50 ft.) in which a good water-supply was always available. They were allowed the ration* usually fed to sheep at this Institute.

At first each sheep was weighed once only, i.e. just prior to slaughter. However, it was later decided to weigh the ewes at weekly intervals, this taking place at 11 a.m. on Monday mornings. The sheep were not starved before being weighed.

Although the ewes were selected at random, they were nevertheless drafted from a flock of fairly uniform size and age. As a result the ewes of the experimental group showed but slight variations in these respects.

Had the weights of the ewes at the time of service been available, it would have been an easy matter to test the group for uniformity of weight. As there were differences in condition (fatness) it cannot be claimed that the weights would have been a true index of skeletal size—the factor which is of importance in the determination of size of the foetus.

It was decided to make use of brain weight as an indication of size. It must at once be pointed out that there is no definite authority for this procedure, and that no undue importance should

* This is as follows:—Roughage always available in racks: ¾ lb. of maize per sheep per day. Green feed or, when this is not procurable, lucerne hay two or three times per week.

be attached to it. However, in the human being it is known that the brain reaches its fullest development relatively early in life (Scammon and Dunn, 1922; Kappers, 1936).

By comparison of the respective life-cycles and the ages of sexual maturity, it is estimated that in the sheep brain weight is likely to have reached its maximum at the age of 2½ years. Therefore, in sheep older than 3 years, but not yet "aged", brain weight should not be affected by small differences in age. Further, it has been shown in the rat that the coefficient of variation of the brain is considerably lower than that of most organs or of the body as a whole (Jackson, 1913). It is evident that from day to day the brain weight of an individual is not likely to change in the same proportion as the body weight which, especially in the ruminant [Bartlett, 1926 (*a*)], is subject to large daily variations. Kappers (1936) states that in the human being body weight and brain weight are correlated. It is felt that these facts go far to justify the use of brain weight as an index of body size.

In Table 1 are given the mean brain weights of the ewes when placed in monthly groups according to the stages of pregnancy reached at the time of slaughter.

When tested by Fisher's (1936) "Analysis of Variance" method these figures show no significant differences.

TABLE 1.

Brain Weights of Ewes.

Groups of Ewes. No.	Class.	No. of Ewes.	Mean Brain Weight.*
1	Non-preg	11	107·73
2	1st month	12	104·42
3	2nd month	8	103·25
4	3rd month	6	106·17
5	4th month	7	107·43
6	5th month	5	104·60

* Weight in Grams.

In Table 2 the conception ages of the same groups of ewes are treated in a similar manner. Again the differences are insignificant. From these tests it is concluded that, although the ewes did show variations both as regards size and age, the effect of the random selection was such as to spread the differences evenly throughout the six groups, there being no "prejudice" in favour of any particular group.

All details regarding dates and times of service and of slaughter are to be found in tabular form in Appendix A.

TABLE 2.

Ages of Ewes.

Groups of Ewes.		No. of Ewes.	Mean age* of Group.
No.	Class.		
1	Non-prog	11	3·72
2	1st month	12	3·75
3	2nd month	8	3·60
4	3rd month	6	3·67
5	4th month	7	3·63
6	5th month	5	3·77

* Age in years.

(b) PROCEDURE.

(1) *Collection of Data.*

Immediately before slaughter the ewe was weighed and a clinical examination was made. Age was determined according to the teeth, while the degree of development of the mammae and of enlargement of the abdomen were determined both by visual inspection and by palpation. Then the animal was placed on its left side and was held firmly while its throat was cut and the spinal cord severed.

Immediately after death the mammary gland was removed; the skin was incised transversely in the region of the umbilicus and the gland was loosened from the abdominal wall; at its caudal border the mammary vessels were examined before being cut through. When the organ, together with its lymph glands, had been freed, the incision through the skin was continued along the circumference of the base. The gland, removed with its covering skin, was laid flat on its base, and left in a cool place until its adipose tissue had set.*

Meanwhile, avoiding rolling of the carcase, assistants had removed the skin and had inserted hooks into the loins, the withers and the neck. By means of these the carcase was supended in imitation of its natural standing posture.

Through slits in the flanks the positions of the rumen, the intestines, the uterus and the ovaries were observed. The gastro-intestinal tract was removed and a note was made of the appearance of the broad ligaments and uterine vessels. Then the entire tract (plus the anus and part of the rectum) was transferred to a mesh-covered frame which fitted accurately over an enamel tray. Various sizes of frame and tray were used, and the weight of each set was known.

* In some instances it was necessary (for the purposes of a different investigation) to inject into the lactiferous sinus a solution of gelatine coloured with Giemsa. The injection was carried out immediately after removal of the gland. The quantity of fluid injected as well as its specific gravity was noted.

The adrenals and the thyroid gland were removed, cleaned of all adherent tissue and weighed. By this time an assistant had opened the skull. The brain was removed in its membranes and with the hypophysis intact. After the medulla oblongata had been severed transversely at the point of divergence of the restiform bodies, the membranes were carefully stripped off and the brain was weighed.* Both the pituitary and the pineal were removed, cleaned and weighed.

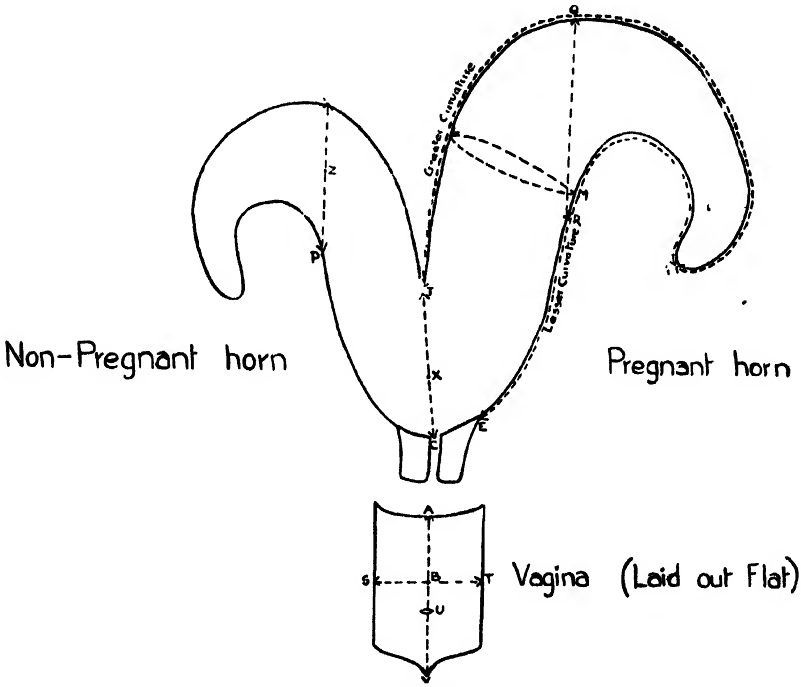


Plate 1.—Showing the method of measuring the uterus and the vagina.

After this the genital tract was again considered. The rectum, the bladder and all loose tissue were removed and the skin and muscular tissue around the vulva were trimmed, care being taken to ensure uniformity in all cases. The vagina and the Fallopian tubes, mesosalpinx and ovaries were detached, thus leaving the " dressed uterus ", the weight of which was then recorded. (In this, as in all similar procedures, the tray was placed on the scale, and the weight of its contents obtained by deducting from the recorded figure the weight of the tray and frame.)

The vagina was weighed and, having been cut longitudinally in the mid-dorsal line, was laid out flat and its length and width were measured as indicated in plate 1. In determining the thickness of the vaginal wall, which was measured at the middle of the length line, the loose adventitious tissue on its outer surface was not included.

* Here the weight of the small piece of bone—dorsum sellae of the sphenoid—which lies between the brain and the hypophysis, was included; however, the relative error is negligible.

Each Fallopian tube, having been freed from its mesosalpinx, was weighed. The length was measured and at the middle of this the diameter was taken.

The ovaries were cleaned and were measured as follows:— Length (in a cranio-caudal direction), width (from side to side) and depth (in the sagittal plane). In each case the maximum measurement was recorded. The presence and appearance of Graafian follicles and corpora lutea were noted and then each ovary was weighed.

Attention was now transferred to the gravid uterus. The broad ligaments were severed in such a way as to allow of the organ being laid out as illustrated in outline in plate 1. Using a piece of twine the lengths of the greater and the lesser curvatures and the circumferences of the horns were measured. (See plate 1.)

Starting from the tip of each horn, and using small blunt-nosed scissors to avoid injury to the underlying sacs, the uterine wall was incised along the greater curvatures. Any strain on the membranes due to their cotyledonary attachments was relieved by cutting through the peduncles of the maternal cotyledons. In this way the uterus was opened completely, leaving the entire foetal system exposed.

At this juncture the situation and the posture of the foetus (visible through the membranes) were noted, as were details of the appearance of the allanto-chorion, the placenta and the uterine mucosa.

After collecting* some allantoic fluid for chemical analysis, the allanto-chorion was slit open and the fluid was allowed to escape. Then the loss of weight was determined and from this and the specific gravity (determined in connection with the chemical investigation) the volume of allantoic fluid was calculated. This was repeated with the amniotic fluid.

After a double ligature had been applied at its foetal extremity, the umbilical cord was severed and the foetus removed. After the adherent mucus and amniotic fluid had been rubbed off its coat the foetus was weighed. Then is was placed on its right side, with its neck and back in the same straight line and with the long axis of the head at right angles to this line.(1) Both the straight and the curved crown-rump lengths were measured (as shown in plate 2). After this the foetus was placed in a flat-bottomed dish in the posture described above and was covered with fixing fluid. For foetuses under the age of 45 days Professor Darts solution (2) was used, while for all others the fluid employed was a 5 *per cent.* solution of neutralized formalin. Foetuses were allowed to remain in this fluid for a few weeks.

* In order to obtain fluid free from any contamination or admixture, it was withdrawn through a hypodermic needle inserted into the sac.

(1) In very young foetuses this was made impossible by the curvature of the body and neck. In these cases the maximum straight measurement was recorded as the straight crown-rump length.

(2) Neutralised formalin ... ... 300 ccm.
Sodium chloride ... ... 75 gm.
Tap water ... ... 9,700 ccm.

The neutralised formalin is prepared from commercial formaldehyde (40 per cent. strength), to 1,000 ccm. of which is added 2 ccm. of a N 10 solution of caustic potash. The precipitate is filtered off through pulp.

By replacement of the membranes upon the uterine wall the detached cotyledons were returned to their original situations. By severing their placental attachments flush with the surfaces of the maternal cotyledons, the membranes were removed and weighed and their volume was determined by the displacement method. It will be noticed that the umbilical cord was included with the foetal envelopes.

After the weight of the empty uterus (i.e. the weight of the uterine wall plus that of the placenta) had been determined, the number of cotyledons in each half of the uterus was counted. For this purpose the line C—J (plate 1) was taken as the division between the two halves.

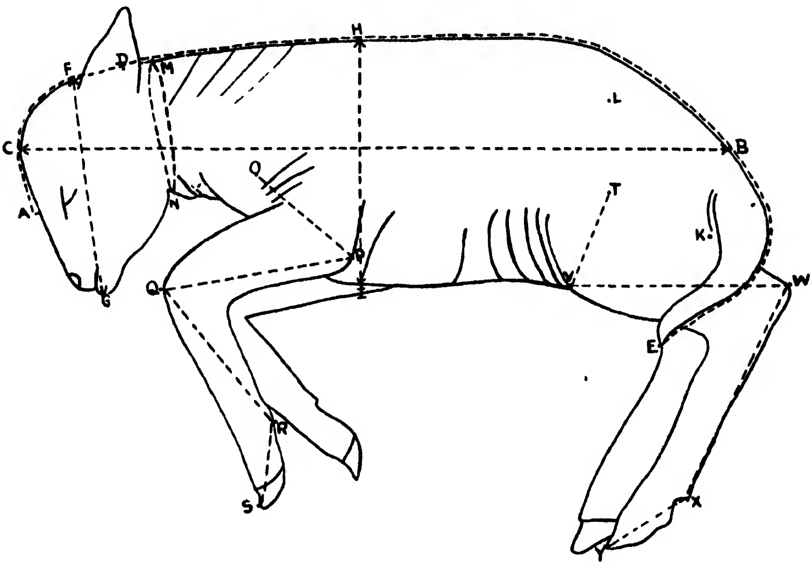


Plate II.—Illustrating the foetal dimensions measured.

The minute, very pale and non-functional cotyledons present in the tips of the cornua were not counted. In each horn the maximum diameter and height both of the largest and of an average-sized cotyledon were measured. In subjects pregnant for 59 days or more the cotyledons were removed from the uterine wall by cutting the mucosal penduncles, and their weight and volume* were determined. Then the uterine wall was weighed, after which it was again stretched out and the cervix was opened by a longitudinal incision. The procedure for determining the length, width and thickness of the cervix was similar to that described for the vagina. Notes were made of the amount and nature of the mucus contained in the cervical canal, while the appearance of the cut surface was also recorded. The length and thickness of the body and the thickness of the wall of each of the horns of the uterus were measured. All the points for measurement are indicated in Plate 1.

* By the displacement method.

By this time the adipose tissue in the mammary gland had set. In removing the covering skin those portions enclosing the teats were left in position. The weight of the gland was determined and where necessary this was corrected for the weight of injection fluid present in the lactiferous sinus.

Later, as time permitted, the preserved foetuses were studied. The crown-rump lengths were re-measured to determine whether shrinkage had occurred. In no instance was any appreciable difference observed. The dimensions listed and illustrated in Plate 2 were measured. Then the external appearance of the foetus was studied, special attention being devoted to the appearance of hair and the development of the appendages.

2. *Treatment of the Data.*

A glance at the tables in the appendix will indicate the variability of most of the data relating to the genitalia, the fluids and the endocrines. In order to minimize this and to arrive at simpler indications of the growth processes involved the data were grouped into six monthly groups according to the stage of pregnancy (the non-pregnant ewes being considered to be 0 months pregnant. The means for the groups were calculated, and these were then tested by Fisher5s (1936) " Analysis of Variance " method, the *Z*-test being employed to determine the existence of significant differences, while the significantly differing groups were picked out by means of the *t*-test.

Here it must be noted that due to the continuous nature of growth the differences within groups are not purely of an experimental nature, hence the accuracy of the tests is impaired. However, as the effect of the error is to obscure significance (by increasing " SD for a single observation ") the results in the present work will not suffer adversely.

As standards for significance were taken the values of Z and t when $P = 0 \cdot 05$ (i.e. 5 per cent. probability) and when $P = 0 \cdot 01$ (i.e. 1 per cent. probability). A positive result at the former level (which already indicates definite significance) is indicated by ×, while a similar result at the higher level of significance is indicated by × ×.

The complete data are given in a series of tables in the appendix. In the text only the means for the groups are mentioned, these figures being employed in the graphs as well as the tables. In the latter are indicated the number of each group, the class (or description), and the number of ewes in the group. Then the mean for the group is stated, and this is followed by the results of the tests for significance. The latter are given in two columns, the first showing the results when each group is tested against the non-pregnant group, while the second indicates the results of testing each group against the one immediately preceding it.

In that section dealing with the foetus the procedure outlined above was not followed. Details of the treatment in this, as well as in a few other special instances, are given at the appropriate places in the text.

The last point to be considered is the method of estimating the age of the foetus. As testing for " heat " was carried out once every twelve hours, the " most probable " time of onset of oestrum was taken to be six hours earlier than the time at which its presence was detected. As the services were regulated so as to ensure the presence of large numbers of active sperms in the Fallopian tubes at the time of ovulation, fertilization was presumed to occur at the thirtieth hour after the onset of heat. It will be seen that this time is determined with greater ease by simply adding twenty-four hours to the time of detection of " heat ". The age of the foetus was calculated from the time of fertilization until the time of death of the mother, and this figure was then approximated to the nearest full day.

CHAPTER 4.—OBSERVATIONS.

(*a*) Genital Tract and Placenta.

1. *Vagina.*

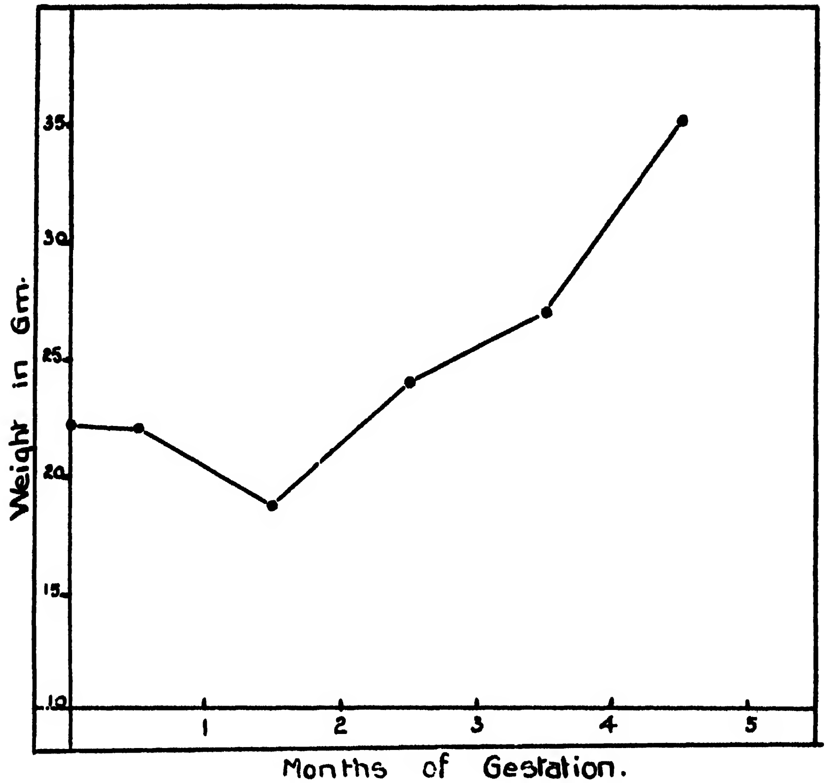
In order to determine whether, during the course of pregnancy, there is definite growth of the vagina, the weight of this organ must be considered. The mean vagina weights in each of the six monthly groups are presented both in tabular and in graphic form (Table 3 and Fig. 1).

Weight of Vagina.

Groups of Ewes.		No. of Ewes.	Mean Vagina Weight.	Significance Tests.	
No.	Class.			W. Group 1.	W. Preced. Group.
			Gram.		
1	Non-preg..........	11	22·45	—	—
2	1st month.........	12	22·25	—	—
3	2nd month.........	8	18·88	—	—
4	3rd month.........	6	24·83	—	—
5	4th month.........	7	27·29	—	—
6	5th month.........	5	35·20	XX	X

The most striking feature of the table is that, apart from the increase in the last month, all the variations are insignificant and may be due entirely to chance. Thus no importance is to be attached to the drop in weight in the second month. When this is disregarded it appears that during the first half of pregnancy the weight of the vagina remains unchanged. From the third month onwards there is to be detected an upward trend which, however, becomes definitely significant in the last month only. By the end of gestation the mean vagina weight has reached a level 50 *per cent.* above that of the non-pregnant group. Of this increase almost 70 *per cent.* is added during the last month, the remainder being accounted for by the small but cumulative increases of the third and fourth months.

Fig. 1.—Weight of Vagina.



Having demonstrated an increase in mass, one may now consider the dimensions of the vagina with a view to determining the manner in which the additional substance is distributed. Length is considered in Table 4 and Fig. 2.

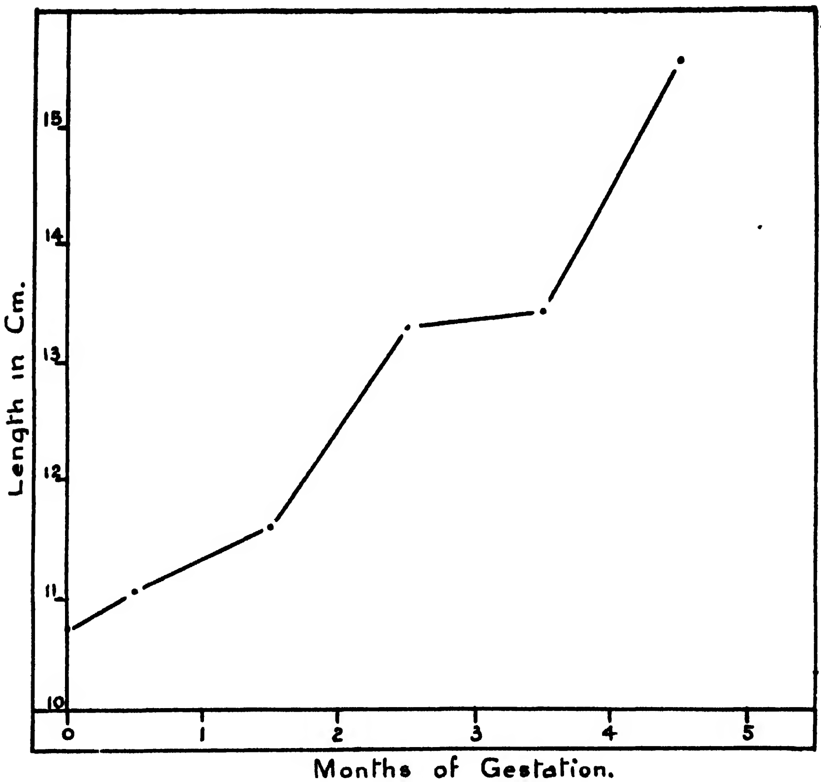
TABLE 4.

Length of Vagina.

Groups of Ewes.		No. of Ewes.	Mean Vagina Length.	Significance Tests.	
No.	Class.			W. Group 1.	W. Preced. Group.
			Cm.		
1	Non-preg..........	11	10·73	—	—
2	1st month.........	12	11·08	—	—
3	2nd month.........	8	11·60	—	—
4	3rd month.........	6	13·33	XX	X
5	4th month.........	7	13·43	XX	—
6	5th month.........	5	15·60	XX	XX

Apart from the absence of a drop in the second month, the general trend of this graph is similar to that of Fig. 1. Again there is little or no change in the first three groups, followed by a definite increase in the following months. However, in its earlier stages the upward trend here is fairly definite, with the result that the total increase, far from being practically confined to the last group, is spread more or less evenly over the last three months.

Fig. 2.—Length of Vagina.



When each group is compared with the one immediately preceding it, it is found that Group 5 does not show significance, whereas both Groups 4 and 6 do. This is to be expected from the marked flattening of the graph between the third and fourth months. For this no reason is apparent, and it might be that it is a discrepancy due to the manner of grouping. It is quite possible that with different grouping the shape of the graph between its two extremities would be altered.

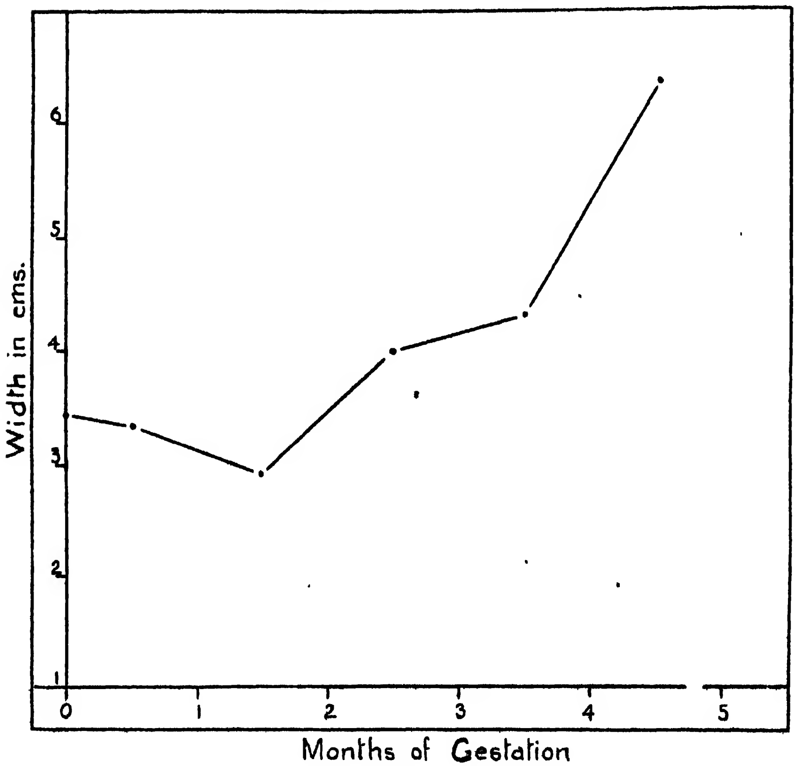
The next dimension to be considered is the width (Table 5 and Fig. 3).

TABLE 5.

Width of Vagina.

Groups of Ewes.		No. of Ewes.	Mean Vagina Width.	Significance Tests.	
No.	Class.			W. Group 1.	W. Preced. Group.
			Cm.		
1	Non-preg..........	11	3·47	—	—
2	1st month.........	12	3·39	—	—
3	2nd month.........	8	2·92	—	—
4	3rd month.........	6	4·00	—	X
5	4th month.........	7	4·30	X	—
6	5th month.........	5	6·40	XX	XX

Fig. 3.—Width of Vagina.



The shape of this graph bears a close resemblance to that of the weight (Fig. 1). The increase in width becomes significant in the last two months only. The positive result obtained when Group 4 is tested against Group 3 is undoubtedly due to the accident of

a low figure in the second month, rather than to a marked increase in the third. As a result of the very steep rise during the last month, the total increase in width amounts to almost 100 *per cent.* of the width of the non-pregnant group.

Fig. 4.—Thickness of Vagina.

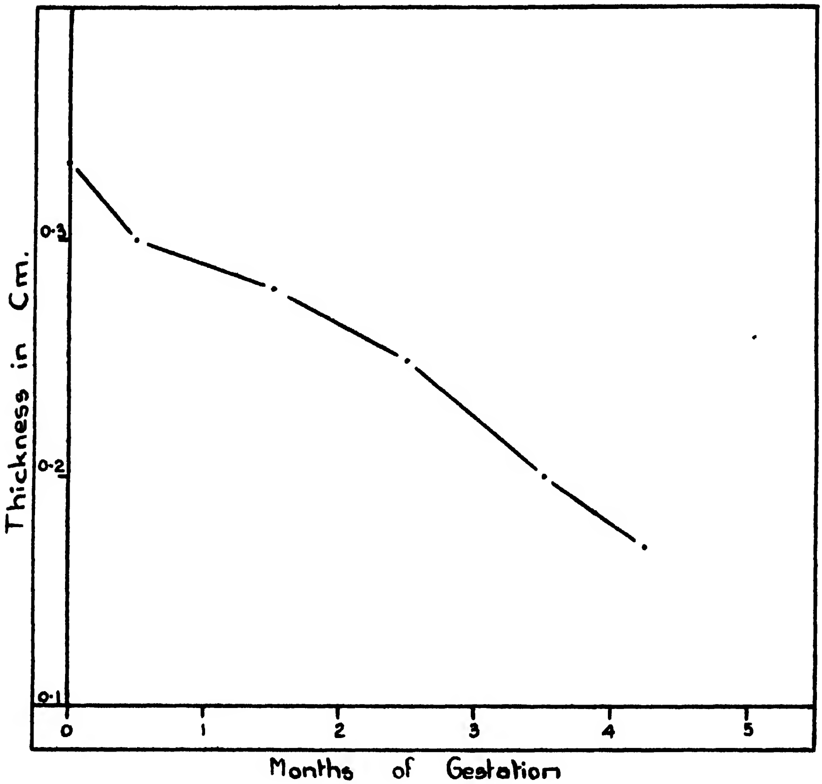


Figure 4 (thickness of vaginal wall) gives the impression that during pregnancy the vaginal wall is reduced steadily to half its original thickness. However, in Table 6 it is seen that not one of the variations is significant, and that all may be due to chance alone. This finding is due to the large degree of variation in the original data (see Appendix), in which consecutive figures in the same group are seen to differ by as much as 400 *per cent.* These widely differing figures are comparatively few. However, under the circumstances, all that can be said is that there is apparently a slight tendency for the thickness of the vaginal wall to be reduced during gestation.

Discussion.

From a consideration of the length and the width of the vagina it becomes evident that, with the advance of pregnancy, the surface area of this organ is increased. This must mean that the lumen of the vagina is enlarged. To a certain extent the reduction in

thickness contributes towards this increase in surface area. However, the main factor is undoubtedly the actual increase of vaginal substance. As there is no internal pressure in the vagina the thinning cannot be due to passive stretching. There must be active re-distribution of the tissue-elements. These processes all indicate a slow preparation of the vagina for the stretching due to occur at parturition. It is presumed that the individual muscle fibres increase in size and become re-distributed to form a thinner layer.

TABLE 6.

Vagina Thickness.

Groups of Ewes.		No. of Ewes.	Mean Vaginal Thickness.	Significance Tests.	
No.	Class.			W. Group 1.	W. Preced. Group.
			Cm.		
1	Non-preg..........	11	0·33	—	—
2	1st month.........	12	0·30	—	—
3	2nd month.........	8	0·28	—	—
4	3rd month.........	6	0·25	—	—
5	4th month.........	7	0·20	—	—
6	5th month.........	5	0·17	—	—

All these changes are recognisable upon macroscopic inspection. The vagina of the non-pregnant ewe is small and compact and ordinarily its lateral walls are in apposition. The labia of the vulva are firm and the opening between them is a mère slit, while the whole of the vaginal lumen is merely a potential space. The mucosa is smooth, only slightly moist and is usually pale. Posterior to the external urethral opening it often has a pinkish-brown colour. During oestrum the mucosa becomes hyperaemic and some clear mucus is present on its surface, while there is a suggestion of increased turgidity of the vulval labia.

For the greater part of the first month of gestation the picture resembles that of the oestral period. Probably this is the effect of the last "heat" period rather than of pregnancy. By the end of the first month the appearance is that of the anoestrál period. This return to the less vascular "inactive" state probably accounts for the slight drops during the second month in Figs. 1 and 3.

Not until the 110th day of gestation is it possible to observe definite departures from this state. At this stage the vagina has become loose and flabby, the labia of the vulva are soft and are no longer in close apposition. From now on these changes become more marked until at the approach of parturition the whole organ is soft and flabby and the lumen is large and fairly patent. In its anterior half a fair amount of white, cloudy and viscid mucus is present, while the mucosa is pale and smooth.

2. *Cervix.*

As the weight of this organ was not recorded separately, it is not possible to determine directly the changes in mass that may occur during the course of gestation. However, these may be deduced from a study of the dimensions of the cervix.

Length is considered in Table 7 and Fig. 5.

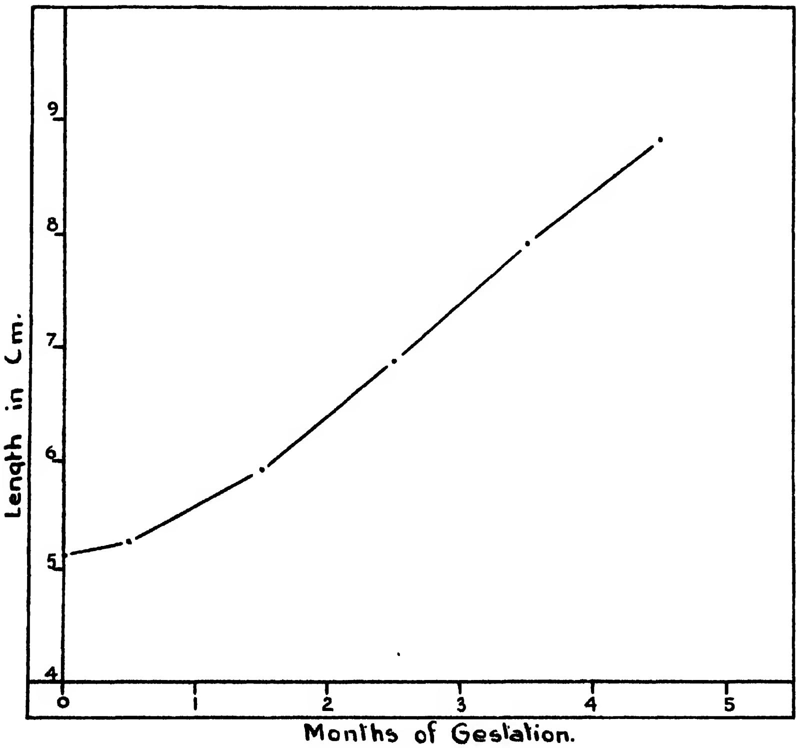
TABLE 7.

Length of Cervix.

Groups of Ewes.		No. of Ewes.	Mean Length of Cervix.	Significance Tests.	
No.	Class.			W. Group 1.	W. Preced. Group.
			Cm.		
1	Non-preg..........	11	5·14	—	—
2	1st month.........	12	5·23	—	—
3	2nd month.........	8	5·94	X	—(1)
4	3rd month.........	6	6·87	XX	X
5	4th month.........	7	7·93	XX	X
6	5th month.........	5	8·80	XX	—(1)

(1) Here significance at P = 0·05 is just missed.

Fig. 5.—Length of Cervix.



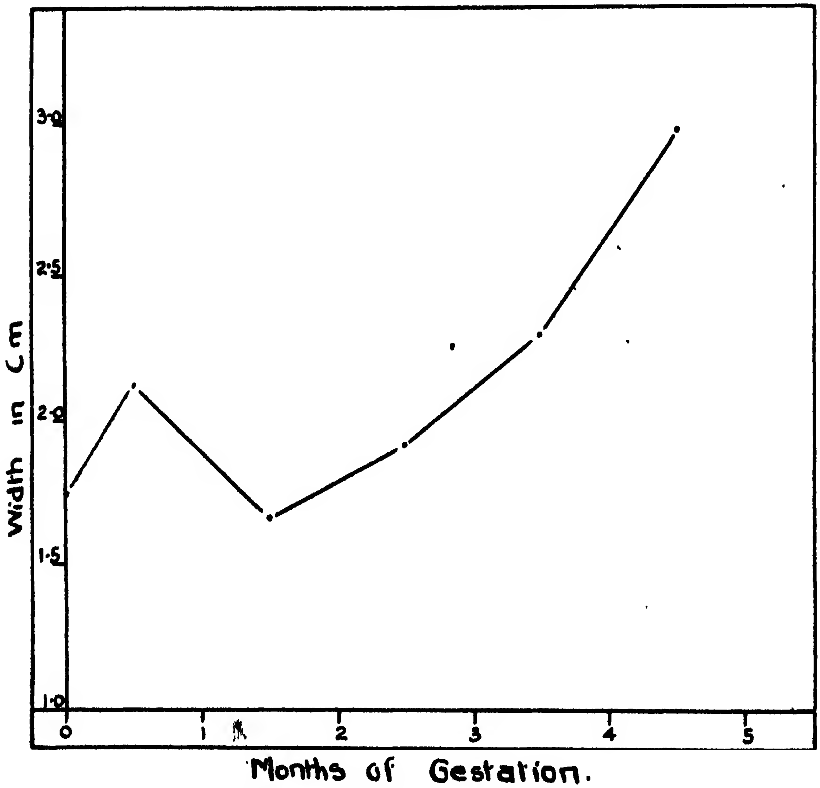
It is seen that there is a definite increase, which is distributed evenly over the entire period of pregnancy. By the second month the increase has become significant and from now on during each month approximately equal amounts are added. This is indicated by the straight line which this portion of the graph assumes.

TABLE 8.

Width of Cervix.

Groups of Ewes.		No. of Ewes.	Mean Width of Cervix.	Significance Tests.	
No.	Class.			W. Group 1.	W. Preced. Group.
			Cm.		
1	Non-preg..........	11	1·75	—	—
2	1st month.........	12	2·12	X	X
3	2nd month.........	8	1·68	—	X
4	3rd month.........	6	1·92	—	—
5	4th month.........	7	2·29	X	—
6	5th month.........	5	2·98	XX	XX

Fig. 6.—Width of Cervix.



The width (Table 8 and Fig. 6) does not exhibit this same regular growth. The peak in the first month is definitely significant. This is followed by a drop in the second month, the figure here being

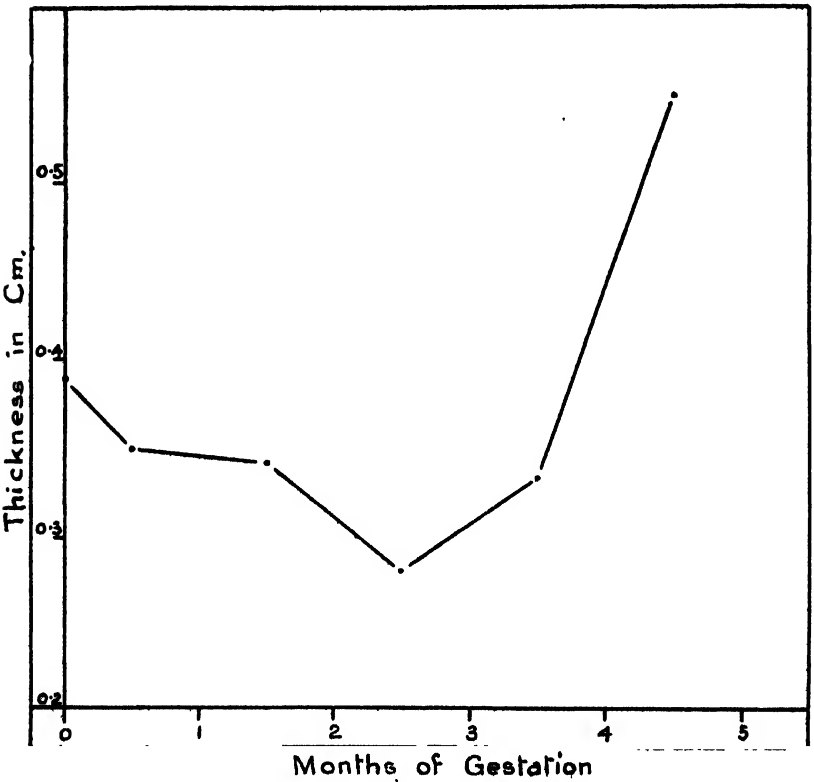
slightly lower than that of group 1. From here onward there is an increase in width, the increment for each succeeding group being larger than that of the preceding one. By the fourth month the width is again significantly larger than that of the non-pregnant group.

TABLE 9.

Thickness of Wall of Cervix.

Groups of Ewes.		No. of Ewes.	Mean Cervical Thickness.	Significance Tests.	
No.	Class.			W. Group 1.	W. Preced. Group.
1	Non-preg..........	11	0·39	—	—
2	1st month.........	12	0·35	XX	XX
3	2nd month.........	8	0·34	XX	—
4	3rd month.........	6	0·28	XX	XX
5	4th month.........	7	0·33	XX	XX
6	5th month.........	5	0·55	XX	XX

Fig. 7.—Thickness of Cervical Wall.



In Table 9 and Fig. 7 are given details of the thickness of the wall of the cervix During the first three months of gestation this dimension decreases until during the third month the lowest level is

reached. In the following two months there are definite increases, that for the last month being very large indeed. This part of the graph rises almost vertically. When compared with the figure of the non-pregnant group all these variations are found to be significant, and in the last column of the table, Group 3 only fails to give a positive result: note the flattening of the graph at this point.

Discussion.

Before discussing these results it is necessary to consider some details concerning the uterine seal, the development of which appears to be closely associated with the changes in the wall of the cervix. Only once (ewe No. 44326 at 130 days) was anything resembling a definite "plug" seen, hence the term "seal" is preferred, as it describes more accuratetly the appearance and distribution of the mucous secretion in the cervix. By the end of the first month of pregnancy small accumulations of mucus may be detected in the depressions between the cervical folds. This is more noticeable in the cranial portions of the organ. As the amount of mucus increases it appears to "glue" together the adjacent surfaces of the folds, and by the 45th day of pregnancy this sealing is complete and a small amount of free mucus is seen in the lumen of the cervix. This tends to collect in the more cranial portions of the organ, where the tissue is less tense and where the folds are small. Gradually the quantity of this cloudy, white, sticky mucus increases, and as this happens the free mucus is no longer found only in the cranial portion, but spreads in a caudal direction. At no stage of pregnancy was the mucus seal found to protrude at the external uterine os.

Whereas the length of the cervix undergoes a steady increase, the width displays a sharp peak, followed firstly by a drop and then by a gradual increase. The first rise may be the result of dilatation of the cervix caused by an increased secretion of mucus during oestrum. As this effect passes off the organ returns to its contracted, dioestral state. Later the effects of pregnancy bring about a steady increase in the width of the cervix. This theory receives support from the fact that in the first month of gestation the thickness of the wall of the cervix is greatly reduced—just what would be expected to result from stretching of the organ caused by pressure in its lumen. As the seal substance accumulates in the cervix the stretching is intensified, hence during the third month there is a further decrease in thickness. After mid-term there is active growth of the organ, with a consequent increase in all dimensions. The thickened wall loses its firmness and becomes soft and slightly oedematous. These changes are first detectable at the 89th day of pregnancy. By this time the accumulation of mucus has increased the size of the lumen, and the whole organ appears enlarged, soft and spongy. At the 110th day these changes are more noticeable and due to the stretching and the accumulation of mucus the folds are well separated. Nine days later the canal is still more dilated and at this stage it is an easy matter to insert a probe into the cervix. At the 130th day the lumen has a diameter of 0·5 cm. and at the 140th day this has increased to 1·0 cm. The whole organ is extremely soft and spongy and it appears markedly oedematous. The tense cervix of the non-pregnant sheep has now become loose and elastic and is capable of extreme dilation.

3. *Body and Cornua.*

From a consideration of its curvatures an indication of the growth in length of the uterus is obtained. In Table 10 and Fig. 8 are to be found details concerning the length of the greater curvatures of both horns.*

TABLE 10.

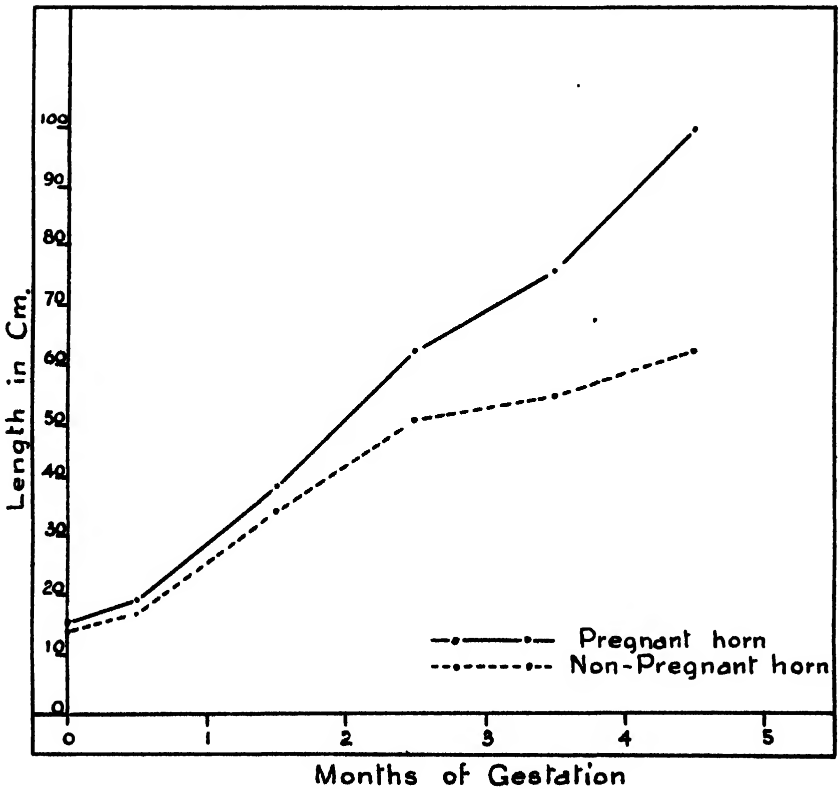
Greater Curvatures of Cornua.

GROUPS OF EWES.

No.	Class.	Ewes.	Preg- nant Horn.	W. Group. 1.	W. Preced. Group.	preg- nant. Horn.	W. Group. 1.	W. Preced. Group.
			cm.			cm.		
1	Non-preg......	11	15·59	—	—	15·18	—	—
2	1st month.....	12	19·12	—	—	17·58	—	—
3	2nd month.....	8	38·06	XX	XX	34·25	XX	XX
4	3rd month.....	6	62·67	XX	XX	50·17	XX	XX
5	4th month.....	7	74·86	XX	X*	54·57	XX	—
6	5th month.....	5	100·00	XX	XX	62·60	XX	X

* Significance at 1 per cent. level is just missed.

Fig. 8.—Greater Curvatures of Cornua.



* Here, and in all similar cases, in Group 1 the left horn has been classed as the gravid one (pregnant 0 days), while the right horn is regarded as being non-gravid at 0 days.

In the pregnant horn there is a steady increase throughout pregnancy. Significance is reached in the second month. The greater curvature of the non-pregnant horn also undergoes much elongation, although its graph always remains below that of the gravid horn. For the first three months the general trend of the two lines is very similar, although from their gradual divergence it is evident that the non-pregnant horn grows at the slower rate. In the last two months the latter curve flattens out considerably, making for much greater dissimilarity between the two curves. The increments for the last two months are much more significant in the gravid horn than in the opposite one (see Table 10—second columns of significance tests).

Table 11 and Fig. 9 show that, regarding the lesser curvatures, there are no marked differences between the two horns. The two graphs assume similar courses, with that of the non-gravid horn always just below the other. The amount of divergence, even at its maximum (in the fourth month), is comparatively small. As is the case with the greater curvatures, here again significance is reached early on in pregnancy, i.e. in the second month.

Fig. 9.—Lesser Curvatures of Cornua.

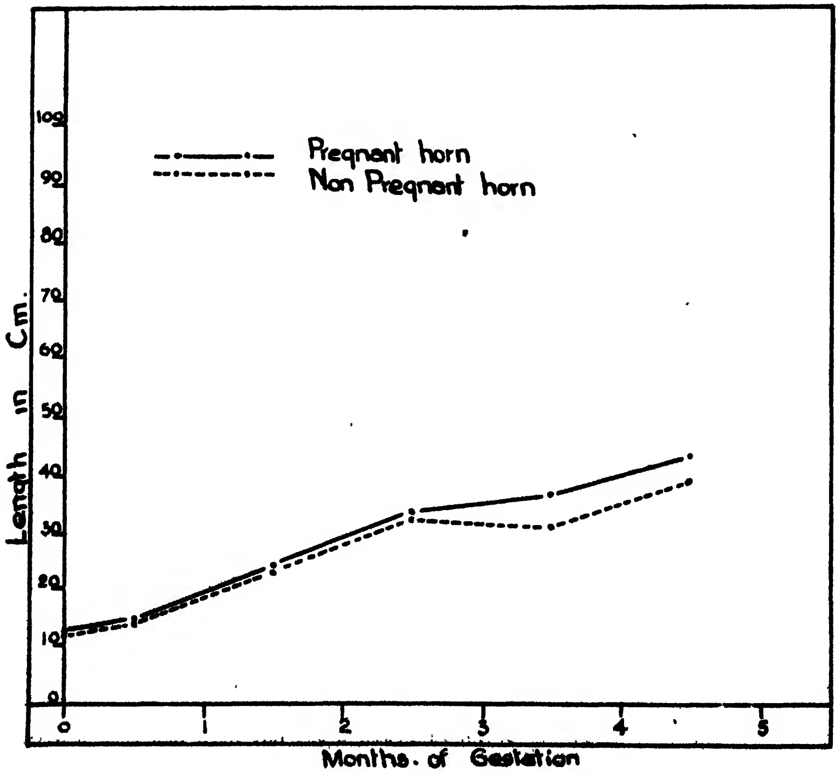
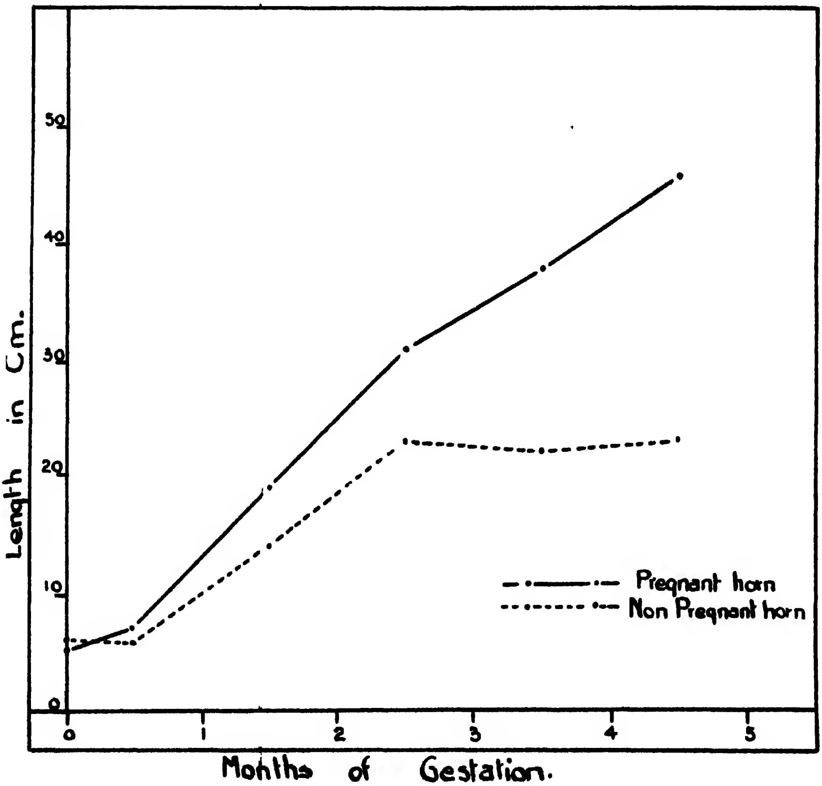


TABLE 11.

Lesser Curvatures of Cornua.

Groups of Ewes.		No. of Ewes.	Mean Length Pregnant Horn.	Significance Test.		Mean Length Non-pregnant Horn.	Significance Test.	
No.	Class.			W. Group. 1.	W. Preced. Group.		W. Group 1.	W. Preced. Group.
			cm.			cm.		
1	Non-preg......	11	11·59	—	—	11·18	—	—
2	1st month.....	12	13·96	—	—	13·63	—	—
3	2nd month.....	8	22·81	XX	XX	22·50	XX	XX
4	3rd month.....	6	33·17	XX	XX	32·83	XX	XX
5	4th month.....	7	36·86	XX	—	31·71	XX	—
6	5th month.....	5	43·80	XX	XX	39·80	XX	XX

Fig. 10.—Circumferences of Cornua.



It appears that in both horns there is considerable growth in length. That there is uneven growth on the two sides of the uterus is evident from the different slopes of the curves in Figs. 8 and 9 (drawn to the same scale). Here it is seen that in each horn the

greater curvature outgrows the lesser, this being particularly noticeable in the last two months. The larger size of the gravid horn is due almost entirely to more extensive development of the greater curvature.

A peculiar feature of both these graphs is the distinct flattening between the third and fourth months. Further, it will be noticed that only in the case of the greater curvature of the pregnant horn is Group 5 significantly larger than Group 4, and even in this instance the level of significance is lower than that of the immediately preceding and following groups. An apparent explanation for this anomalous behaviour will suggest itself later, when the volumes of the foetal fluids are studied.

There is a fairly close resemblance between the graphs of the circumferences (Fig. 10) and those of the greater curvatures (Fig. 8). Again the line for the non-gravid horn lies below the other and diverges from it, slowly up to the third month and then more rapidly. After the third month the circumference of the non-pregnant horn does not increase at all, whereas during the same time the monthly increments of the gravid horn remain definitely significant (Table 12). Here too the peculiar flattening of the curve, referred to above, is evident. In the pregnant horn it is of only slight degree, yet in the other horn it is most marked.

TABLE 12.

Circumferences of Cornua.

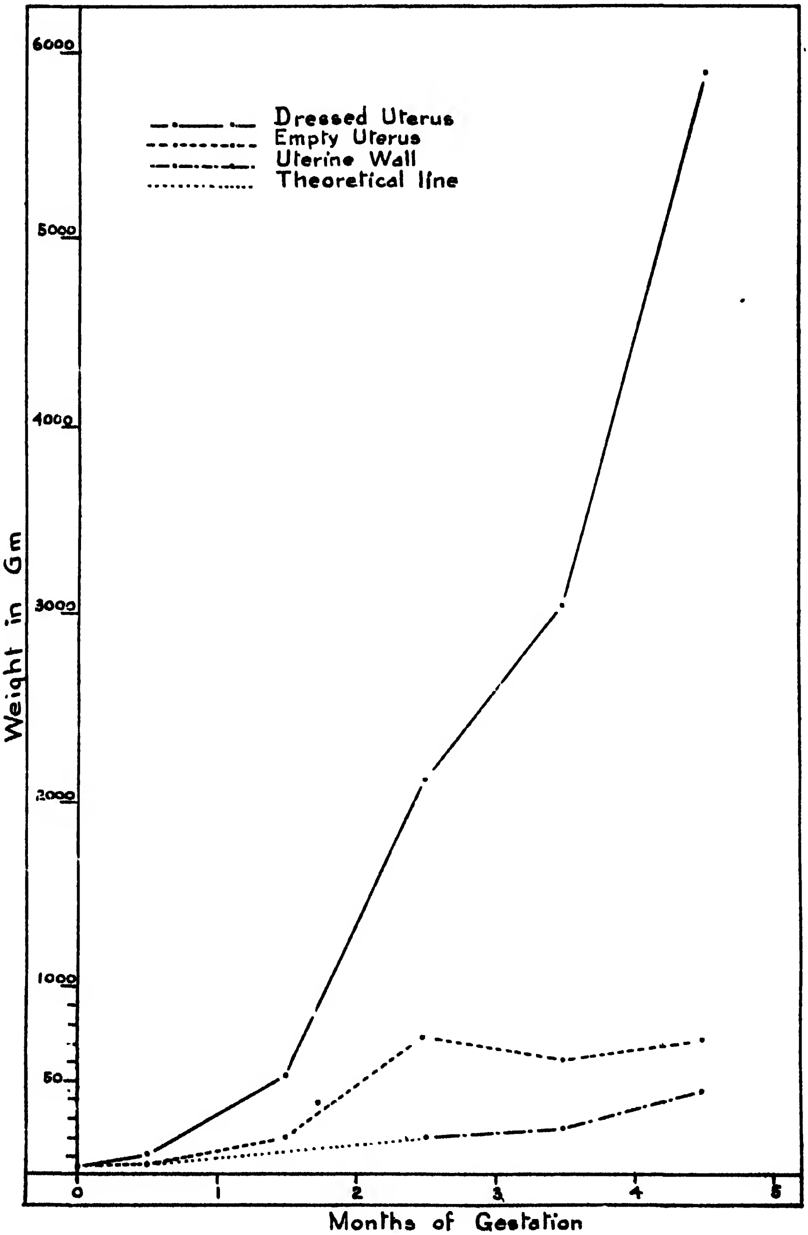
Groups of Ewes.		No. of Ewes.	Mean Length Pregnant Horn.	Significance Test.		Mean Length Non-pregnant. Horn.	Significance Test.	
No.	Class.			W. Group. I.	W. Preced. Group.		W. Group. I.	W. Preced. Group.
			cm.			cm.		
1	Non-preg......	11	5·05	—	—	5·23	—	—
2	1st month	12	6·87	—	—	5·73	—	—
3	2nd month.....	8	18·75	XX	XX	14·00	XX	XX
4	3rd month.....	6	31·08	XX	XX	23·00	XX	XX
5	4th month.....	7	37·57	XX	XX	22·00	XX	—
6	5th month.....	5	45·49	XX	XX	23·00	XX	—

Discussion.

It is only to be expected that with the advance of pregnancy there will be an increase in the size of the uterine horns. Here it is seen that during the first half of gestation both horns increase fairly steadily, with but a slight difference in favour of the pregnant horn. However, during the second half of pregnancy the horn in which the foetus lies completely outgrows the other, this being noticeable in both curvatures (not marked in the lesser) as well as in circumference. As a result of the uneven distribution of the

increase, the enlargement of the horns is accompanied by a change in shape. All these changes are well illustrated in the photographic plates presented by Curson and Quinlan (1934) and Curson and Mare (1934).

Fig. 11.--Weights of Dressed Uterus, Empty Uterus and Uterine Wall.



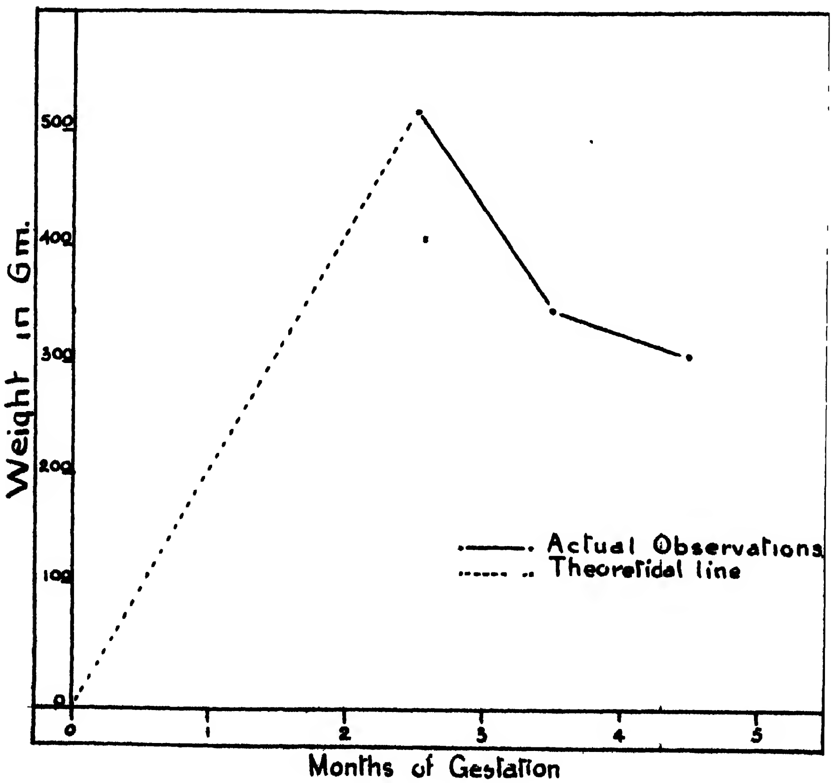
The growth of the uterus in weight is considered in Table 13 and Fig. 11. By the end of pregnancy the "dressed" uterus has reached the relatively enormous weight of approximately 6 Kg.

TABLE 13.

Weights of Dressed Uterus, Empty Uterus and Uterine Wall.

Groups of Ewes. No.	Groups of Ewes. Class.	No. of Ewes.	Mean Weight Dressed Uterus.	Significance Test. W. Gr. 1.	Significance Test. W. Prec. Gr.	Mean Weight Empty Uterus.	Significance Test. W. Gr. 1.	Significance Test. W. Prec. Gr.	Mean Weight Uterine Wall.	Significance Test. W. Gr. 1.	Significance Test. W. Prec. Gr.
			Gm.			Gm.			Gm.		
1	Non-preg..	11	42·73	—	—	42·73	—	—	42·73	—	—
2	1st month.	12	65·92	—	—	39·67	—	—	?	?	?
3	2nd month.	8	519·75	X	X	202·75	XX	XX	?	?	?
4	3rd month.	6	2,128·67	XX	XX	740·17	XX	XX	221·00	XX	?
5	4th month.	7	3,026·57	XX	XX	597·43	XX	X	248·00	XX	—
6	5th month.	5	5,880·80	XX	XX	708·80	XX	—	442·00	XX	XX

Fig. 12.—Weight of Placenta.



The graph rises but little in the first month, more in the second (at this stage the increase becomes significant) and then assumes an exceedingly steep slope. Between the third and fourth months there is a distinct flattening of the curve. Later it will be seen that this is caused in part by a decrease in the total volume of foetal fluid and in part by a drop in placental weight.

The weight of the empty uterus is the sum of the weights of the uterine wall and the placenta. Details of the latter are presented in Table 14 and Fig. 12. For the first four months of pregnancy the course of the graph of the empty uterus is governed mainly by the weight of the placenta. Until the end of the second month there is a gradual rise, and then in the third month there is a sudden peak, followed by a drop. Meanwhile the weight of the uterine wall has increased steadily, but this has not been sufficient to influence to any great extent the weight of the empty uterus. In the last month there is a highly significant increase in the weight of the wall and as a result of this the graph of the empty uterus rises again to the level of the third month, in spite of the fact that at this stage the placental weight undergoes a further (but insignificant) reduction.

Fig. 13.—Length of Body of Uterus.

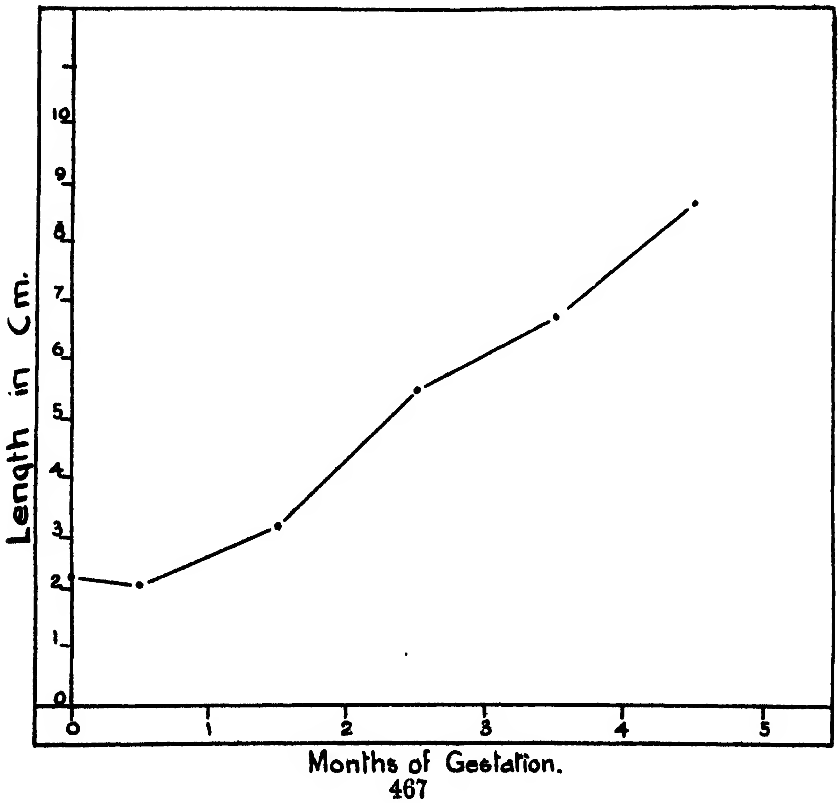


TABLE 14.

Weight of Placenta.

Groups of Ewes.		No. of Ewes.	Mean Weight of Placenta.	Significance Tests.	
No.	Class.			W. Group 1.	W. Preced. Group.
1	Non-preg..........	11	Nil	—	—
2	1st month.........	12	?	—	—
3	2nd month.........	8	?	—	—
4	3rd month.........	6	519 gm.	XX	?
5	4th month.........	7	349 gm.	XX	X
6	5th month.........	5	307 gm.	XX	—

The increase in size of the uterus is not limited to its horns, but is also evident in *the body*. When it is remembered that the body is the portal through which the cornua communicate with each other, it is not surprising to find that in its increase in length (Table 15 and Fig. 13) this portion of the uterus follows a course closely resembling, and more or less intermediate between, those observed in connection with the greater curvatures of the cornua. Here too a slight flattening of the curve in the fourth month of pregnancy, is observed.

TABLE 15.

Length of Body of Uterus.

Groups of Ewes.		No. of Ewes.	Mean Length of Body.	Significance Tests.	
No.	Class.			W. Group 1.	W. Preced. Group.
			Cm.		
1	Non-preg..........	11	2·16	—	—
2	1st month.........	12	2·07	—	—
3	2nd month.........	8	3·19	*	X
4	3rd month.........	6	5·50	XX	XX
5	4th month.........	7	6·71	XX	XX
6	5th month.........	5	8·70	XX	XX

* Just misses significance at 5 per cent. level.

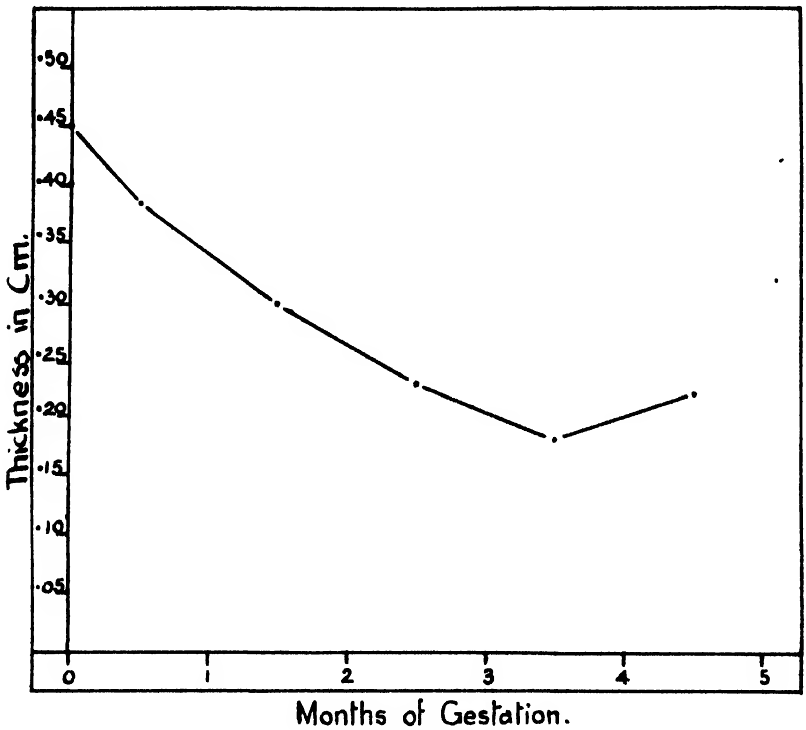
From the fact that by the end of the second month of gestation the body is not yet* significantly longer than in the non-pregnant group, it is concluded that in this part of the uterus growth is initiated later than in the horns. In this connection it is to be

* By how little significance is missed is indicated by the fact that Group 3 is significantly larger than Group 2, which is but slightly lower than Group 1.

observed that during the first month there is no change from the non-pregnant state, in fact the mean of Group 2 is slightly (but not significantly) lower than that of Group 1.

In each of the three portions of the uterus (the two horns and the body) the *thickness of the wall* was measured. Details are presented in Table 16. In the column headed " Total Groups " are tabulated the means of all the measurements recorded in each month, while in the last line of the table are given the " Averages " for all the readings (throughout pregnancy) at each point.

Fig. 14.—Thickness of Uterine Wall.



When these data are analysed, by means of Fisher's Z-test, to show the effects of (*a*) stage of gestation (total groups), (*b*) position (in pregnant or non-pregnant horn or in body), and (*c*) interaction between stage of gestation and position, a positive result is obtained in the first instance only. From this it is concluded that the effect of pregnancy is to cause a reduction in the thickness of the uterine wall, that this influence is exerted evenly over the whole wall, and that in their response to this effect all three parts behave identically.

The significance tests referred to in Table 16 are those concerning the " total groups ". These indicate that as early as the first month of gestation there is a significant reduction of the thickness of the uterine wall. In each of the following three months a further significant decrease occurs, so that by the end of the fourth month the

uterine wall has been reduced to one-third of its original (non-pregnant) thickness. In the last month there is an insignificant increase in thickness. This is caused solely by the high figures obtained at the 147th day, resulting from the general oedematous condition of all the genitalia at this stage.

TABLE 16.

Thickness of Uterine Wall.

Groups of Ewes.		No. of Ewes.	Thickness of Uterine Wall.			Total Groups.	Significance Test.	
No.	Class.		Body.	Preg. Horn.	Non-preg. Horn.		W. Gr. I.	W. Preced.
			Cm.	Cm.	Cm.	Cm.		
1	Non-preg......	11	·45	·45	·46	·45	—	—
2	1st month.....	12	·39	·38	·38	·38	XX	XX
3	2nd month....	8	·29	·30	·30	·30	XX	XX
4	3rd month.....	6	·23	·22	·23	·23	XX	XX
5	4th month.....	7	·20	·17	·18	·18	XX	X
6	5th month.....	5	·27	·18	·22	·22	XX	—
	AVERAGE......	·33	·31	·32				

Discussion.

The small, pale and contracted uterus of the non-pregnant ewe changes during pregnancy. By the eighteenth day the horns appear slightly puffy and by its slightly larger size it is possible to distinguish the horn in which the ovum has become implanted. At about this time the colour of the organ changes to a distinct pink and numerous very tortuous subserous bloodvessels become visible. As gestation progresses so these vessels become larger and less tortuous, the colour becomes redder, and the size-difference between the horns becomes much more distinct. After the 38th day there is also a change in the shape of the horns, this being the result of more rapid growth along the greater curvatures. Seven days later the uterine wall has a bluish-grey colour and the subserous vessels are large and pursue an almost straight course. The largest are to be found along the lesser curvatures, and from these branches run across the uterine horns at right angles to their long axes. By this time the walls have become markedly thinner and through them the outlines of the cotyledons may be distinguished. From the middle of gestation the pregnant horn grows much more rapidly than its fellow and the discrepancy in size becomes so marked that towards the end of the fourth month the non-gravid horn has become merely an appendage of the other. At this stage the walls have become very thin and vascularity is pronounced throughout the organ. This state is maintained until the approach of parturition. At the 147th day the thickness of the wall is increased as a result of a general oedema of the genitalia.

In the non-pregnant uterus the mucosa is pale and but slightly moist. Early in pregnancy there is a slight reddening at the site of implantation and this spreads rapidly until at the 45th day the entire mucosa is markedly vascular. At this stage the epithelial lining appears to have been destroyed (at least the surface is not smooth and clear) and the surface is covered with a layer of dirty, greyish, sticky material. As a result of this the allanto-chorion is found to adhere to the inner surface of the uterine wall and in stripping it off care has to be exercised not to rupture the sac. This condition persists until midway through the fourth month, after which the epithelial lining appears to be restored, and with the disappearance of the slimy coating the mucosa is again smooth, fairly moist and of a light red colour At this stage the amount of adherence between the uterus and the foetal sac is negligible

4. *Placenta.*

It has been seen (Table 14 and Fig. 12) that the weight of the placenta increases to a peak in the third month, after which it drops fairly rapidly. In an attempt to determine the cause of this behaviour the numbers and the sizes of the cotyledons have been studied. Details of the former are presented in Table 17 and Fig. 15.

Fig. 15.—Number of Cotyledons.

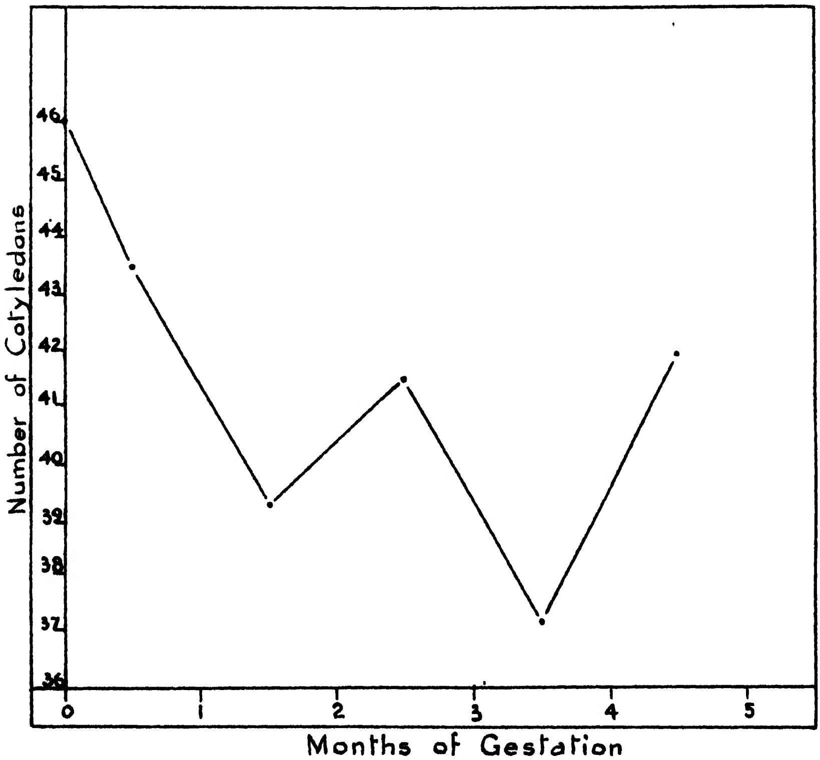
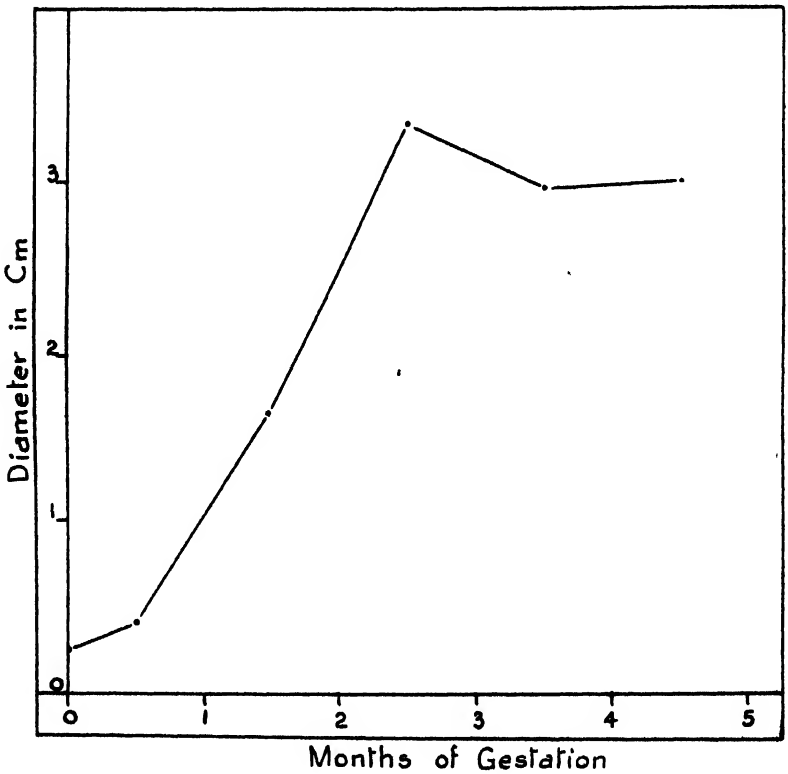


TABLE 17.

Number of Cotyledons.

Groups of Ewes.		No. of Ewes.	Mean Number of Cotyledons.		Total Groups.	Significance Tests.	
No.	Class.		Preg- nant Side.	Non- preg. Side.		W. Group, 1.	W. Preced. Group.
1	Non-preg.	11	46·64	45·26	46·00	—	—
1	1st month.............	12	44·17	42·92	43·54	—	—
3	2nd month.............	8	42·13	37·63	39·38	XX	X
4	3rd month.............	6	43·83	39·33	41·58	X	—
5	4th month.............	7	37·71	36·57	37·14	XX	X
6	5th month.............	5	43·00	40·80	41·90	—	X
	AVERAGE.........	—	43·31	41·02	...		—

Fig. 16.—Diameter of Cotyledons.



When these data are analysed in the manner described in connection with the thickness of the uterine wall, it is found that there is a significant difference between the pregnant and non-pregnant sides of the uterus. The excess in favour of the former side is never great,

and falls far short of the figure (50 per cent.) mentioned by Bergmann (1922) in his work on the bovine. From the tests it appears that there are significant differences at the various stages of pregnancy. Here it must be remembered that in the first two groups it was impossible to distinguish between functional and non-functional cotyledons, thus the figures given represent counts of all the cotyledons as compared with counts only of functional cotyledons in the remaining groups. Hence it is not surprising to find that the numbers for the first two groups are higher than those of any other group. As this method of testing is open to criticism, it is more satisfactory to employ Group No. 3 (the first in which functional cotyledons are easily recognisable) as the standard for comparison. When this is done it appears that in spite of a fair degree of variation in the last four groups, none of the differences are significant. Therefore, the differences observed may be ascribed to "unfair" counts in the first two groups, and it may be concluded that, whatever the true position regarding functional cotyledons in the first month (in Group 1 their number is nil), the observed variations in the weight of the placenta are not caused by variations in the numbers of cotyledons.

TABLE 18.

Diameter of Cotyledons.

Groups of Ewes.		No. of Ewes.	Mean Diameter of Cotyledons.		Total Groups.	Significance Tests.	
No.	Class.		Preg-nant Side.	Non-preg. Side.		W. Group 1.	W. Preced. Group.
			Cm.	Cm.	Cm.		
1	Non-pregnant..........	11	·28	·30	·28	—	—
2	1st month......	12	·42	·39	·40	—	..
3	2nd month........ ...	8	1·86	1·47	1·67	XX	XX
4	3rd month.............	6	3·45	3·24	3·35	XX	XX
5	4th month.............	7	3·04	2·94	2·99	XX	X
6	5th month.............	5	3·00	3·00	3·00	XX	—
	AVERAGE.........	—	1·63	1·52	—	—	—

As a result of the above conclusion, it becomes necessary to consider the size of the cotyledons at the various stages of pregnancy. For this purpose two dimensions (diameter and height) have been studied. In each instance the average of the readings for the largest cotyledon in the horn and one representing the modal size of that horn, is employed. Details of these representative figures are presented in Tables 18 and 19 and Figs. 16 and 17. Both these dimensions increase rapidly (becoming significant in the second month) up to the end of the third month. After this their behaviour differs, the diameter dropping significantly in the fourth month and then maintaining its level in the fifth, whereas the height still increases (although insignificantly) in the fourth month and then undergoes a significant decrease in the last month of pregnancy. However, by

the end of gestation both dimensions are significantly lower than they were in the third month. It is clear that the placental weight is affected mainly by this change in the size of each individual cotyledon.

Fig. 17.—Height of Cotyledons.

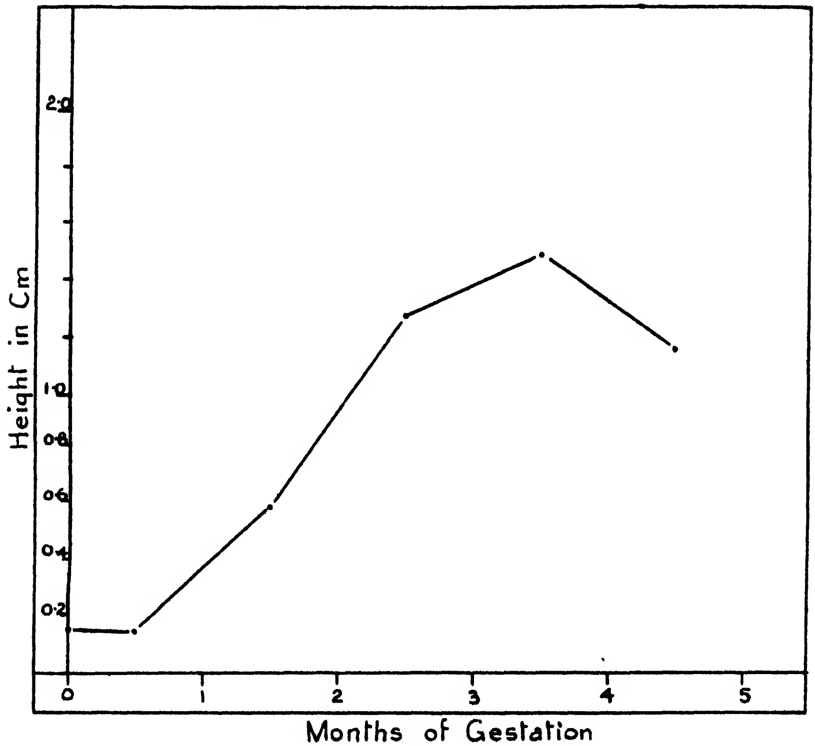


TABLE 19.

Height of Cotyledons.

Groups of Ewes. No.	Groups of Ewes. Class.	No. of Ewes.	Mean Height of Cotyledons. Pregnant Side.	Mean Height of Cotyledons. Non-preg. Side.	Total Groups.	Significance Tests. W. Group 1.	Significance Tests. W. Preced. Group.
			Cm.	Cm.	Cm.		
1	Non-pregnant..........	11	·15	·16	·16	—	—
2	1st month.............	12	·16	·15	·15	—	—
3	2nd month.............	8	·63	·55	·59	XX	XX
4	3rd month.............	6	1·42	1·37	1·39	XX	XX
5	4th month.............	7	1·50	1·46	1·48	XX	—
6	5th month.............	5	1·16	1·16	1·16	XX	XX
	AVERAGE.........	—	·681	·656	—	—	—

Discussion.

It has been seen that during pregnancy the cotyledons increase enormously both in diameter and in height. Apart from this enlargement there are other changes which may be observed macroscopically. In the non-pregnant uterus the cotyledons appear to be arranged in four longitudinal rows. All are of the same size and are just visible as pinhead-sized brownish centres with pale peripheral zones. In dioestrum they are level with the surface of the mucosa, but during oestrum they become slightly swollen and elevated. Early in pregnancy a change is evident and by the 21st day both the size of the central portion and the elevation of the cotyledon are visibly increased. During the next few days the brownish centre, previously concave, becomes flat and numerous pin-point red spots appear all over its surface. On the 27th day the cotyledon has the form of a small nodule under the epithelial layer of the uterus. Four days later the central portion has a distinct red colour and a pitted appearance, while the periphery has become elevated to form a thin circular lip around the wide flat centre. From this time until the 80th day the cotyledon develops rapidly: it enlarges and the peripheral lip begins to curl inward, thus decreasing the size of the central cup and trapping the foetal cotyledon which has come to rest upon the surface of the cup. At the same time the base of the cotyledon becomes convex and detaches itself from the surrounding tissue to such an extent that finally the entire cotyledon is attached to the uterine wall merely by a thin mucosal peduncle.

At about the end of the third month the cotyledons appear to have reached their maximum development. The cups are small and the rolled-in edges resemble motor-tyres in miniature. In some instances the cups tend to lose their circular shape and become elongated slits. From this stage onward fairly large blood clots of long standing are visible in the cups of the cotyledons. Apart from a tendency to decrease somewhat in size (partly due to a tighter rolling in of the edges) the cotyledons do not undergo further change until about the 140th day, after which the turned-in periphery relaxes and the whole cotyledon opens out flat with the central portion partially everted and covered with clotted blood. By the 147th day most of the cotyledons resemble flattened reddish-black discs, while in a few the eversion has been so marked as to give the appearance of a mushroom (similar to the convex cotyledon of the bovine). At this stage the attachment of the membranes is not firm and they may be pulled away with ease. Usually the entire central portion of the cotyledon comes away, leaving an empty thin-walled cup.

In the above description only the general appearance of the cotyledons at each stage has been mentioned. However, at one and the same time a variety of phases of development may be encountered. This is well illustrated in the accompanying plate (No. III). Development is initiated at the site of implantation and throughout the progressive stage the largest and best developed cotyledons are to be found here. In the opposite horn growth is slower and the maximum is reached later. In both horns the largest cotyledons are found towards the centre, while the size decreases towards the apices and towards the body. In the latter only a few cotyledons are present and

these are but poorly developed. During the last two months there is practically no difference between the cotyledons of the pregnant and non-pregnant horns, although the differences at different situations in the horn persist until parturition. The smaller cotyledons, although slower in growth, appear to reach the same stage of differentiation as the largest ones.

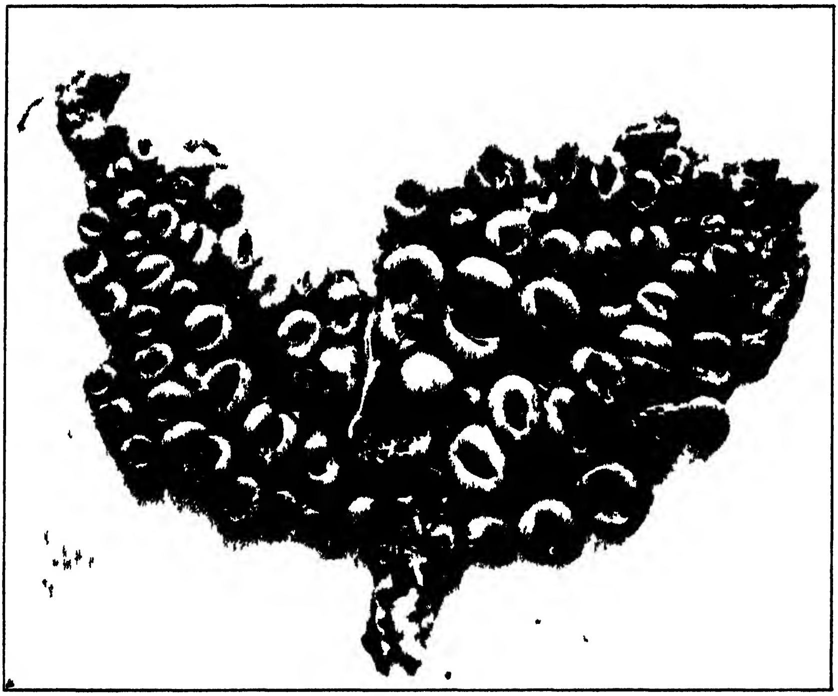


Plate III.—Gravid uterus laid open to show cotyledons, pregnant horn on the right and cervix towards the bottom.

The foetal part of the placenta also develops fairly early. At the 21st day of pregnancy dull white areas appear on the allanto-chorion wherever it touches the surface of the maternal cotyledons. A few days later these patches have become almost opaque and at the 31st day numerous closely grouped pin-point red spots mark out on these areas the size and shape of the central portions of the maternal cotyledons. At this time there is a very loose attachment of membrane to cotyledon. The red spots increase in size and coalesce, and small vessels can be distinguished running from these circumscribed areas to the umbilical cord. By the 45th day these areas have developed into thick discs firmly attached to the outer surface of the allanto-chorion. By this time there is a fair degree of adherence between the discs and the maternal cotyledons. From now on the edges of the latter curl inward to hold the foetal cotyledons firmly in position, thus completing the cotyledonary attachment.

5. *Fallopian Tubes.*

The tubes were considered from the point of view of length, diameter and weight. For each of these characteristics an analysis similar to that described in connection with the thickness of the uterine wall, has been undertaken and the results are presented in Tables 20-22.

TABLE 20.

Length of Fallopian Tubes.

Groups of Ewes. No.	Class.	No. of Ewes.	Mean Length. Pregnant Side.	Mean Length. Non-preg. Side.	Total Groups.	Significance Tests. W. Group 1.	Significance Tests. W. Preced. Group.
			Cm.	Cm.	Cm.		
1	Non-pregnant....	11	15·41	15·05	15·23		
2	1st month....	12	15·55	15·88	15·72		
3	2nd month....	8	18·00	17·13	17·56	XX	XX
4	3rd month	6	17·17	17·08	17·13	X	
5	4th month	7	17·64	17·57	17·61	XX	
6	5th month...... .. .	5	18·60	17·90	18·25	XX	
	AVERAGE.........		16·73	16·35			

TABLE 21.

Diameter of Fallopian Tubes.

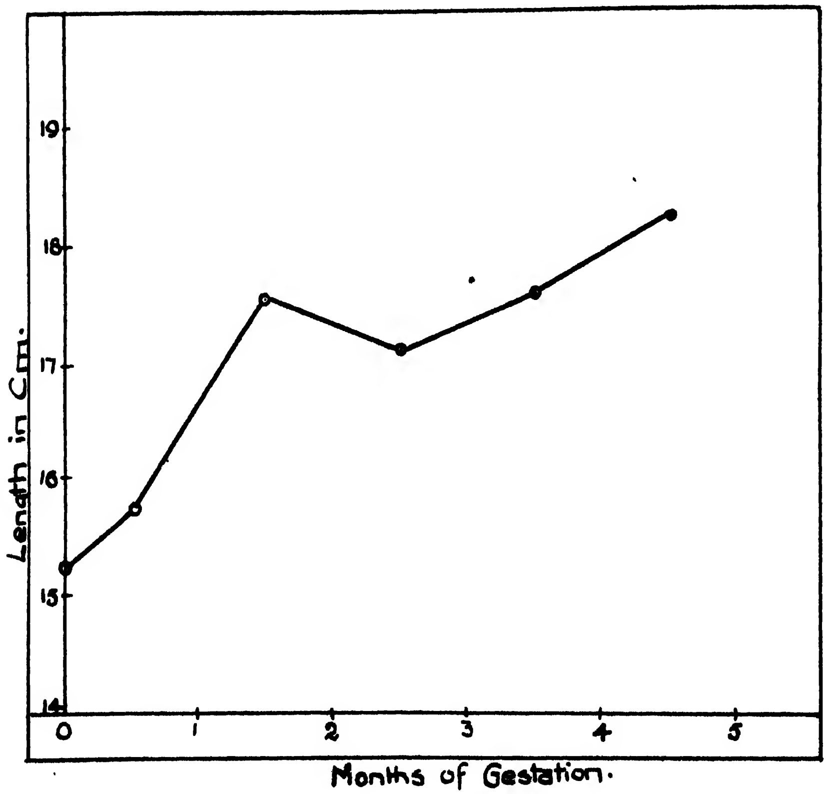
Groups of Ewes. No.	Class.	No. of Ewes.	Mean Diameter. Preg-nant Side.	Mean Diameter. Non-preg. Side.	Total Groups.	Significance Tests. W. Group 1.	Significance Tests. W. Preced. Group.
			Cm.	Cm.	Cm.		
1	Non-pregnant..........	11	·21	·21	·21		
2	1st month............	12	·19	·20	·20		
3	2nd month...........	8	·19	·19	·19		
4	3rd month............	6	·19	·19	·19		
5	4th month............	7	·19	·19	·19		
6	5th month............	5	·20	·20	·20		–
	AVERAGE.........	-	·196	·196	–	—	-

TABLE 22.

Weight of Fallopian Tubes.

Groups of Ewes.		No. of Ewes.	Mean Weight.		Total Groups.	Significance Tests.	
No.	Class.		Preg-nant Side.	Non-preg. Side.		W. Group 1.	W. Preced. Group.
			Gm.	Gm.	Gm.		
1	Non-pregnant..........	11	0·5227	0·4773	0·5000	—	—
2	1st month.............	12	0·4858	0·5042	0·4950	—	—
3	2nd month.............	8	0·5438	0·5375	0·5406	—	—
4	3rd month.............	6	0·4750	0·4617	0·4683	—	—
5	4th month.............	7	0·5423	0·5100	0·5172	—	—
6	5th month.............	5	0·5000	0·5200	0·5100	—	—
	AVERAGE.........	—	0·5092	0·5008	—	—	—

Fig. 18.—Length of Fallopian Tubes.



In no instance is it possible to demonstrate any difference between the tube from the pregnant side and the opposite one. However, when the total groups are considered, significant variations are encountered. Here the last four groups are significantly longer than the first two, but show no real differences among themselves. This is illustrated in Fig. 18. From the second month of gestation the tube from the pregnant side is always slightly (but not significantly) longer than the other.

Discussion.

As the increase in length is not accompanied by an increase in weight, actual growth cannot account for the elongation. It is suggested that a certain amount of stretching of the tube occurs early in pregnancy when both curvatures of the horn are growing steadily. The reason why this stretching does not appear to cause a decrease in the diameter of the tube may be that the latter was measured at the middle of the length, whereas the stretching is likely to occur mainly at the uterine end of the tube. If mechanical stretching is the cause of the elongation, then the latter cannot be regarded as a true change of pregnancy, and it must be concluded that it is not possible to demonstrate that gestation has any effect on the macroscopic appearance of the Fallopian tubes.

(*b*) Foetal Membranes and Fluids.

1. *Membranes.*

The weights tabulated in Table 23 are those of the two membranes combined. These data are presented graphically in Fig. 19. The reason for the omission of Group 1 is obvious.

The membranes grow rapidly until the end of the third month; in the following month there is hardly any increase in weight, and then in the last month the rate of increase exceeds that of any of the previous months. Attention is directed to the flattening of the graph during the fourth month, this being similar to what is observed in connection with the growth of the uterus.

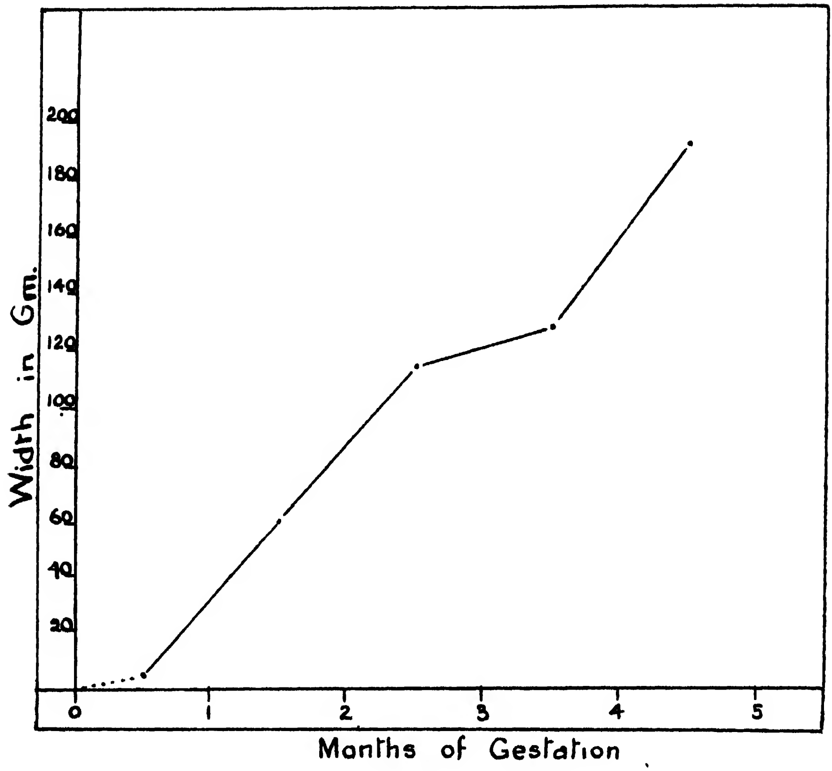
Table 23.

Weight of Foetal Membranes.

Groups of Ewes.		No. of Ewes.	Mean Weight of Membranes.	Significance Tests.	
No.	Class.			W. Group 2.	W. Preced. Group.
			Gm.		
2	1st month.........	7	6·94	—	—
3	2nd month.........	8	60·70	X	X
4	3rd month.........	6	115·58	XX	X
5	4th month.........	7	128·57	XX	—
6	5th month.........	5	192·80	XX	X

As at all times the volume of the membranes is very similar to their weight, the data for the former have not been analyzed.

Fig. 19.—Weight of Foetal Membranes.



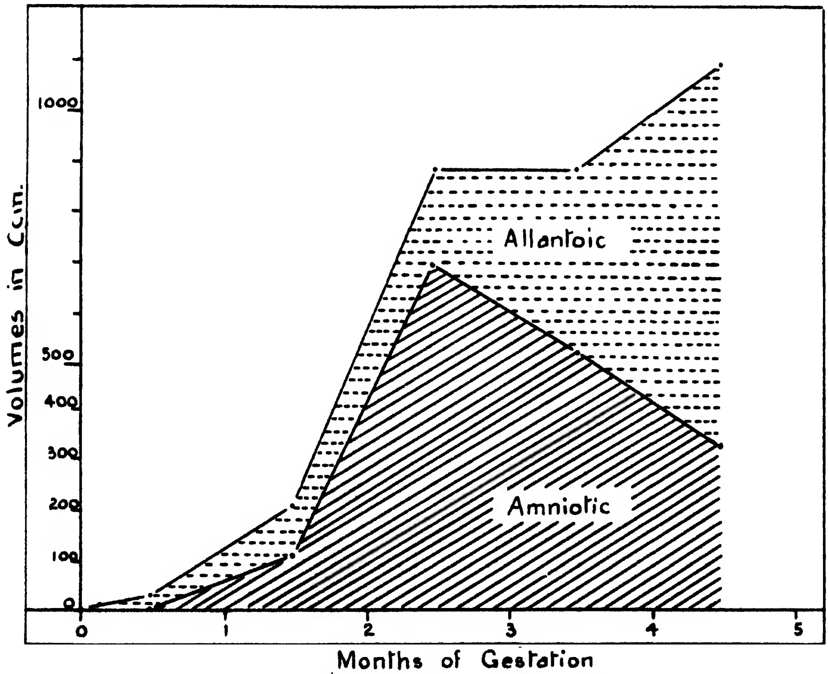
Discussion.

It has been seen that the membranes undergo an enormous increase in weight during pregnancy. However, the two membranes do not at all times contribute equally to this enlargement. At the 16th day of pregnancy an elongated pale membrane is seen in the pregnant horn, but it is not till the 18th day that the two sacs can be distinguished. At this time the allantois is distended with fluid and it extends almost the entire length of the gravid horn. The amnion is just visible as a narrow crescent-shaped membrane closely investing the embryo, which is situated towards the middle of the length of the allantoic sac. Two days later the latter sac has extended into the body of the uterus and some distance along the non-gravid horn. At this stage this sac appears to be composed of a loose, velvety outer layer and a clearer inner one in which the blood vessels seem to be located. Of the latter there are two main sets, extending from the umbilical region to each tip of the sac, and from these small branches run across the inner surface of the sac. By the 25th day the narrow, elongated allantoic sac has reached almost to the apices of both uterine horns. At this time the amnion begins to enlarge slightly, but it is not until the 30th day that it stands away clearly from the foetus.

From now on the membranes increase in size and also in thickness. The bloodvessels enlarge and their branches become more numerous. At the middle of the second month opaque white flakes begin to appear on the surface of the allantoic membrane. These are seen first in the region over the foetus, from where they spread towards the tips. They are never so dense as to impair seriously the transparency of the membrane. Now too the necrotic tips of the allanto-chorion are plainly visible. At the 66th day the amnion has enlarged to such an extent as to bring the central zone of its outer surface into direct contact with the overlying parts of the chorion. Thus the allantoic sac is divided into two compartments which communicate with each other and with the urachus by means of a narrow tube-like cavity.

Until the 100th day both sacs are well filled with fluid, hence the the membranes are tense. After this time the same degree of distension is apparently not maintained, so that by the end of the fourth month the sacs appear slightly collapsed. Especially is this the case with the amnion. The membranes are now relatively thick and fairly strong. Till the end of gestation they remain colourless and more-or-less transparent.

Fig. 20.—Volumes of Foetal Fluids.



(Note.—Allantoic Volume is represented by the *vertical* distance between the two lines.)

2. *Foetal Fluids.*

Details of the volumes of both the allantoic and the amniotic fluids are presented in Table 24. In Fig. 20 the upper curve represents the total volume of fluid while the lower one indicates

the volume of amniotic fluid. The allantoic volume is represented by the portion lying between the two graphs. The total volume increases rapidly until the end of the third month. This is caused mainly by the amniotic fluid, which reaches its peak in the third month. Although the volume of allantoic fluid increases during this period, the rate is comparatively slow. However, in the fourth month this rate is accelerated to such an extent that the increase in the allantoic fluid is sufficient to neutralize the effect of a rapid drop in the amniotic volume, and thus in this month the total volume remains practically unchanged. In the last month the allantoic fluid is doubled and as a result of this the total volume shows a distinct rise, in spite of the continued decrease in amniotic fluid.

TABLE 24.

Volumes of Foetal Fluids.

Groups of Ewes.		No. of Ewes.	Mean Vol. Allantoic.	Significance Tests.		Mean Vol. Amniotic.	Significance Tests.	
No.	Class.			W. Group 1.	W. Preced. Group.		W. Group 1.	W. Preced. Group.
			cc.			c.c		
2	1st month.....	9	24·17	—	—	1·5	—	—
3	2nd month.....	8	93·63	—	—	112·69	X	X
4	3rd month.....	5	185·20	X	—	698·00	XX	XX
5	4th month.....	7	347·29	XX	—	525·71	XX	XX
6	5th month.....	5	762·00	XX	XX	329·00	XX	XX

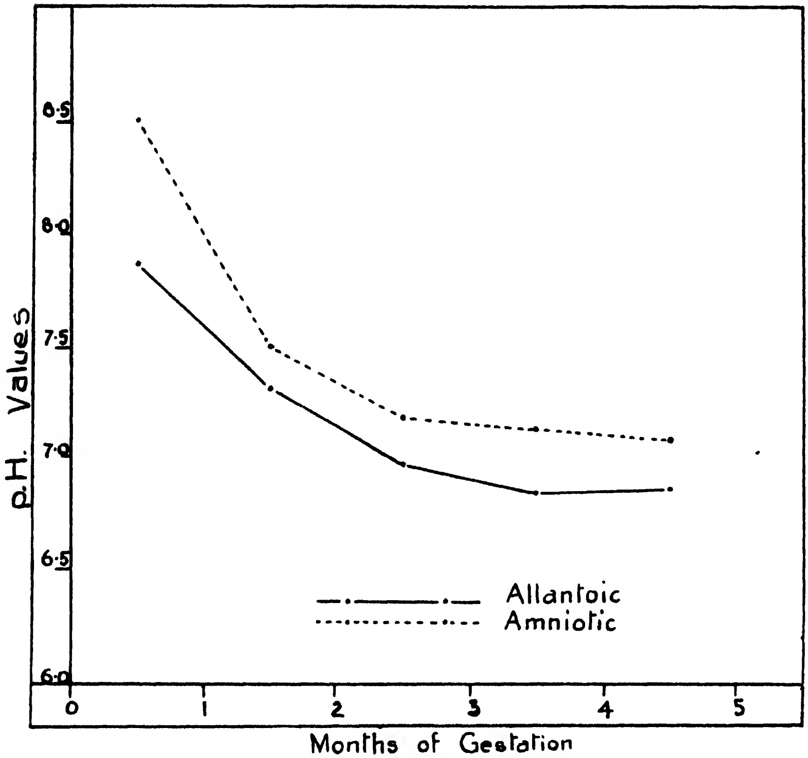
In Table 25 it is seen that, as regards hydrogen ion concentration, there are differences between the two fluids; also in each fluid this concentration decreases with the advance of gestation. However, in both fluids this reduction is significant only in the second month, after which the variations are slight. These facts are illustrated in Fig. 21.

TABLE 25.

Hydrogen Ion Concentration of Fluids.

Groups of Ewes.		No. of Ewes.	Mean pH. Allantoic.	Significance Tests.		Mean pH Amniotic.	Significance Tests.	
No.	Class.			W. Group 1.	W. Preced. Group.		W. Group 1.	W. Preced. Group.
2	1st month.....	6	7·867	—	—	8·500	—	—
3	2nd month.....	8	7·329	XX	XX	7·500	XX	XX
4	3rd month.....	5	6·960	XX	—	7·180	XX	—
5	4th month.....	7	6·829	XX	—	7·143	XX	—
6	5th month.....	5	6·860	XX	—	7·080	XX	—

Fig. 21.—Hydrogen Ion Concentration of Foetal Fluids.



The specific gravity of the fluids is considered in Table 26. Due to insufficient data for the first month, Group No. 2 had to be omitted. All that may be concluded from these figures is that the specific gravity of the allantoic fluid is always higher than that of the amniotic. All other variations, even the apparent steady downward trend in the case of the amniotic fluid, are insignificant.

TABLE 26.

Specific Gravities of Fluids.

Group of Ewes.		No. of Ewes.	Mean Specific Gravity.	
No.	Class.		Allantoic.	Amniotic.
3	2nd month...............	6	1·0075	1·0045
4	3rd month...............	5	1·0123	1·0042
5	4th month...............	7	1·0127	1·0043
6	5th month...............	5	1·0114	1·0026

Discussion.

In the failure of the total volume of foetal fluid to increase during the fourth month of pregnancy lies the explanation for the peculiar flattening observed in most of the curves dealing with growth of the uterus and its parts. Apparently to some extent enlargement of these parts is dependent upon the stretching effect of the foetal fluids. Moreover, at this time, the fluids constitute a very large proportion of the weight of the entire foetal system. These facts make it clear why in its growth the uterus should follow a course resembling closely that of the increase in volume of the fluids.

Both as regards hydrogen ion concentration and specific gravity the fluids differ one from the other. In other physical properties these differences are also recognizable. In the early stages of pregnancy both fluids are clear, colourless and watery. This is maintained until the 45th day, at which time the allantoic fluid turns slightly cloudy. During the following two weeks this turbidity becomes more pronounced and a lemon tinge appears in the previously colourless fluid. This becomes more intense and then gradually changes to amber. At the 81st day it is difficult to decide which of the two colours is present, but a week later there is no longer any doubt. The bright amber colour then seen darkens into a golden brown, which shade is encountered throughout the remainder of the period. The turbidity previously mentioned increases until the end of the third month, as does the viscosity. During the last two months the fluid again becomes watery, clear and practically transparent.

In the amniotic fluid the lemon tinge is visible at the 52nd day. Within a short time it has changed to amber, but the latter shade is never very deep. During the fourth month the amber colour disappears and the fluid acquires a very light green colour. In the meantime the viscosity and the turbidity have increased so that by the end of the fourth month the amniotic fluid is thick and "syrupy" and very cloudy. The colour varies from a pale green to a dirty white. During the later stages a fair amount of meconium is found in the amniotic fluid.

(c) Foetus.

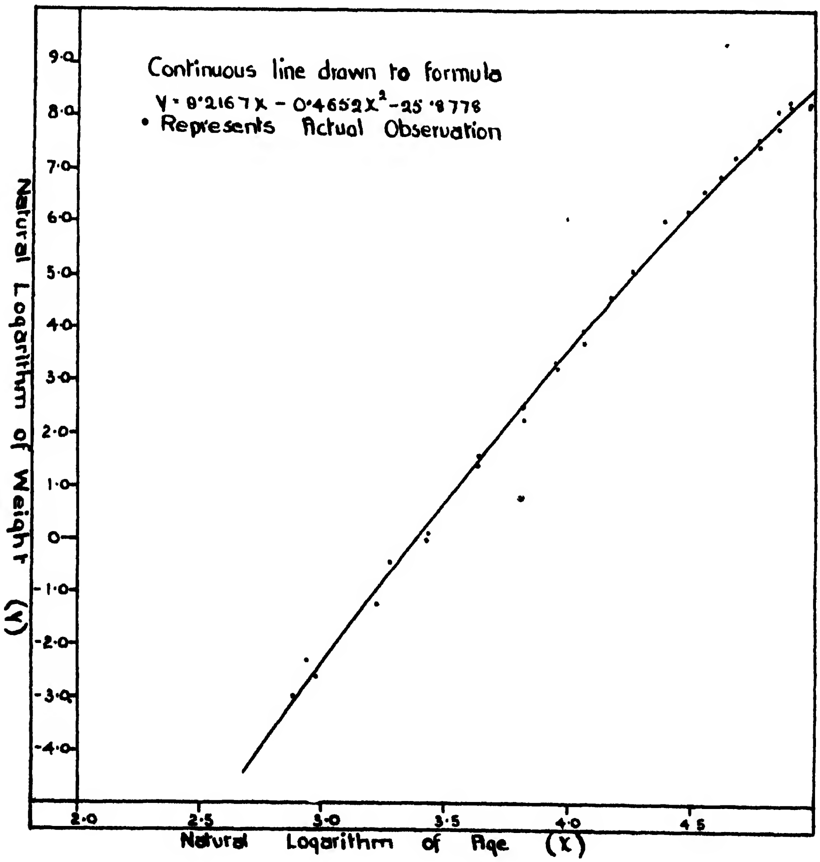
All details regarding age, weight and dimensions of the entire series of foetuses are presented in tabular form in Appendix A. The trends of the growth processes exhibited by these observations are best studied through the process of curve-fitting. In order not to complicate the text with statistical technicalities all details of the procedure adopted have been relegated to Appendix B.

1. *Growth in Weight.*

MacDowell *et al* (1927) maintain that when for a series of foetuses the natural logarithms of age are plotted against those of the corresponding weights, a straight line distribution results. However, in their work age is calculated not from the moment of

fertilization of the ovum, but from the time of formation of the "embryo proper".* So distinct is the linear trend of the data in Fig. 22 that one is led to surmise that, in spite of the difference in the method of computation of age, here too the general type of MacDowell's formula will hold good. Moreover, it is possible to fit such a line to the data and to obtain a result significant at 1 per cent. probability. Nevertheless, the points representing the actual observations are not evenly scattered about this straight line. When, by the use of antilogarithms, a weight-age curve is constructed from the formula, it becomes quite evident that the formula in question does not give a good representation of the trend of the data. Towards the latter end of the period the curve rises much too steeply and thus passes well to the left of all the actual observations.

Fig. 22.—Natural Logarithms of Weight plotted against those of Age.



* Probably the stage with the primitive axis established and with the formation of somites just commencing.

In view of the above it becomes necessary to test the "fit" of a parabola. The regression formula obtained is:—

$$\text{Log}_e \text{ Wt} = 9{\cdot}2167 \text{ Log}_e \text{ Age} - 0{\cdot}4652 (\text{Log}_e \text{ Age})^2 - 25{\cdot}8778,$$

and the continuous line in Fig. 22 is drawn from it. Both factors b_1 and b_2 (see Appendix B) are highly significant (at $P = {\cdot}01$), while the coefficient of variation is 20·07 per cent., which is 4 per cent. lower than that of the straight-line formula. Both in Fig. 22, and in the weight-age curve in Fig. 23, the actual data are well scattered around the curves, indicating that the formula from which these curves are constructed gives a true representation of the trend of the data. The figure for the coefficient of variation (20 per cent.) is still high, but, in view of the large variations in the original data (see differences in weights of foetuses of identical age) this is only to be expected. Fitting to a further degree will certainly not bring about any improvement and will only serve to complicate the formula.

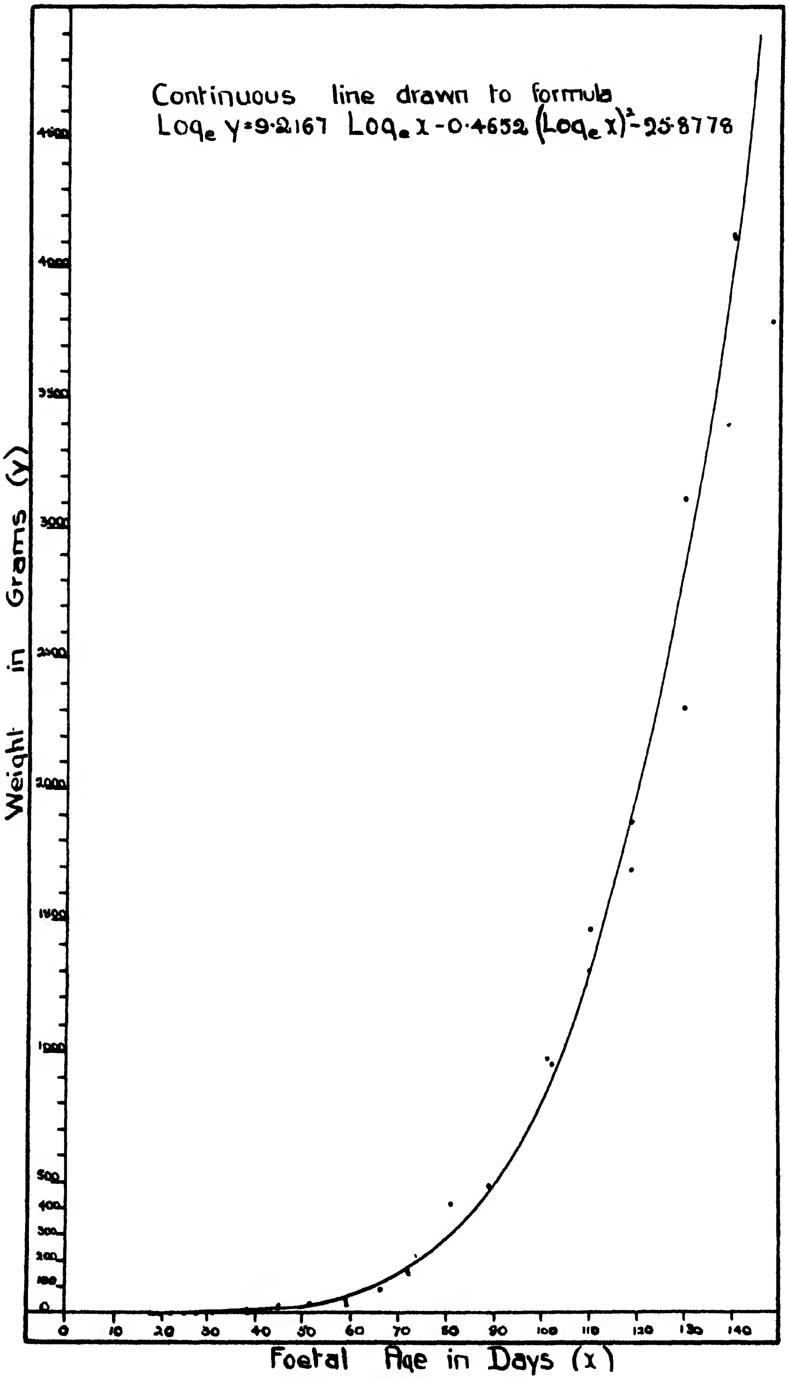
It is concluded that the age and weight data obtained from the series of Merino foetuses are best fitted by a curve of the second degree. This type of formula produces a curve with two inflections, the first convex, and the second concave to the axis. In Fig. 23 the second inflection is not distinct. Nevertheless the retarding effect of the factor "0·4652 (Log_e Age)^2" is evident in the widening and flattening of the curve during the latter part of prenatal life.

The presence of the second inflection is of importance; nevertheless it has either not been recognised or its significance has not been appreciated and has thus not been stressed. It has been customary to look upon the prenatal growth curve as one continuously rising and joining on to the initial part of the postnatal curve without any significant break. Now, however, it becomes apparent that, at least in the case of the Merino foetus, the prenatal growth curve has both accelerated and retarded phases and that, if these two phases together constitute a "growth-cycle", then it must be concluded that one such cycle is completed before the birth of the lamb.

It has been shown by Scammon and Calkins (1923) that growth in length of the human foetus is best represented by a curve of the second degree. This appears to support the above conclusions and thus suggests that the growth of all foetal dimensions follows a similar trend. This surmise will be tested later in this work.

Scammon (1922) maintains that there is no sudden change in growth at the time of birth, but that the transition is gradual. Kislovsky and Larchin (1931) state that in the bovine there is little difference between the relative growth-rates just before and just after birth. From the above it is to be concluded that there are no abrupt changes of direction of the curve at the time of birth. It is a feature of the curve described that the second inflection makes it possible to link up pre- and post-natal curves in a manner which satisfies the above conditions.

Fig. 23.—Growth in Weight.



It must be stressed here that thus far absolute growth has been under consideration. The curves mentioned are of a cumulative nature and indicate at each age the summation of all the gains up to that stage. They illustrate the course of growth in weight. For comparative purposes it is desirable to be able to express these values as relative or percentage figures. In order to avoid the fallacious computations so often encountered in works of this nature, it is essential to remember that, by virtue of the constantly changing mass of the growing foetus, it is impossible to arrive at a true determination of the percentage growth-rate through any calculation based upon the "simple interest" conception. The errors introduced by such methods are exposed by Brody (1927*a*), who shows that the rates mentioned by Minot (1908) are all too high and that the exaggeration is greatest when the rate is highest and when the time interval between successive observations is long.

The growth process is best likened to the increase of capital set out at interest which is being compounded instantaneously, i.e. the increment for one instant forming part of the capital for the following instant. However, the rate of interest (which is the percentage growth-rate) does not remain fixed, but is subject to change during the course of prenatal life.

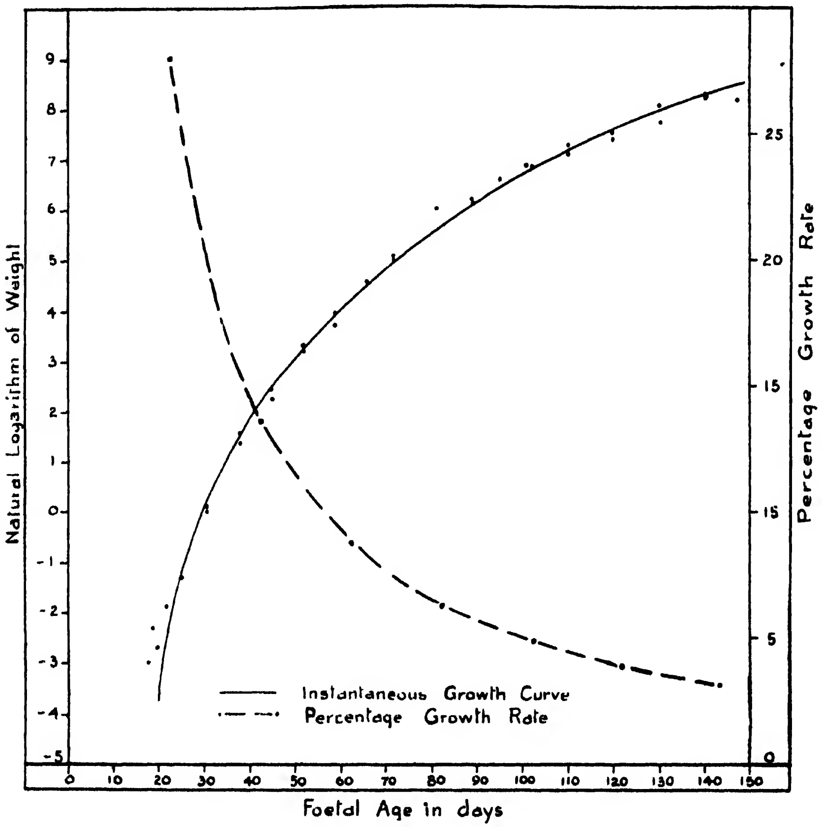
Brody (1927*a*) appears to have been the first investigator to appreciate these facts. By recourse to the calculus he is able to demonstrate that the graph resulting from the plotting of the natural logarithms of weight against age actually represents the trend of the instantaneous growth-rate, and that the slope of the curve at any point is an indication of the growth-rate at that moment.

From the data at his disposal Brody (1927*b*) concludes that such instantaneous growth-rate curves are composed of several segments set at varying and decreasing slope. Thus he maintains that the growth-rate remains steady over a considerable period and then changes abruptly to a lower level.

In Fig. 24 is presented the curve for instantaneous growth-rate of the Merino foetus. The dots represent the actual observations while the continuous line is drawn through points obtained by plotting weight values calculated from the formula previously mentioned. It is observed that the resulting graph is a smooth curve concave to the axis of the figure. This is contrary to Brody's findings, for here there is no evidence of straight segments. Here the decrease in the rate of growth is spread evenly over the entire period and is not limited to certain very short and well defined intervals. It is hardly likely that the difference in these findings is attributable to the species on which the observations have been made. Not only does Brody's work cover a wide range (the rat, the guinea-pig, the chick and man) but also regarding the bovine similar results are claimed by Kislovsky and Larchin (1931). On the other hand, in their work on the mouse MacDowell *et al* (1927) present a curve almost identical with that in Fig. 24. There is much point in these authors' criticism of Brody's work, namely that the data on which he relied were unsatisfactory and that he failed to realise that any curve may be approximated by a series of straight lines.

Both Brody and Kislovsky and Larchin merely plot their data on arithlog paper and then draw their straight lines by inspection. Brody (1927a) actually claims for this method the special merit of keeping the normal trend of the data more prominently under consideration, as compared with the tendency in the method of least squares of stressing the deviations from this normal trend. However, his arguments do not appear to affect the present situation, in which both the actual data and the "fitted" data are plotted. When it is observed that the numbers of observations in Brody's and in Kislovsky and Larchin's works are limited, and that there are fair deviations from the actual lines drawn, then it must be realised that their conclusions are open to criticism.

Fig. 24.—Curves showing Instantaneous Growth-rate and Average Percentage Rate of Growth in Weight.



It is significant that in the present investigation and in MacDowell's work, where efforts have been made to limit the factors likely to cause variations in foetal weight and where relatively large numbers of observations have been recorded, smooth curves are obtained. Further, it is apparent from Fig. 25 that when the chance deviations from the general trend are eliminated then the plotted points all fall directly on a smooth curve.

It must be concluded that the weight of evidence is against the occurrence of abrupt " breaks " in the growth-rate. In the sheep definitely (and most probably in other mammals too) the rate decreases in a steady and regular manner throughout the course of prenatal life.

The instantaneous relative growth-rate (designated K) may be calculated on a daily basis from the following formula:—

$$K = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1}$$

where W_2 and W_1 are the foetal weights at the ages T_2 and T_1 days respectively.

The figure obtained is an average value over the period T_1 to T_2 days. As it has been noted that the rate of growth is changing continuously, the value obtained will vary somewhat with the length of the period $(T_2 - T_1)$. The amount of variation will not be large. However, for comparative purposes it will be better to keep the period constant for all such calculations.

By multiplying the value of K by 100, the rate may be expressed as a percentage, thus:—

Instantaneous (Average) Percentage rate per day $= 100 \times K$

$$= \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1} \times 100$$

In Table 27 are tabulated such percentage rates calculated over periods of five days at twenty day intervals throughout the foetal period.

TABLE 27.

Growth-Rates.—Per Cent. Per Day.

Age.	Value of K.	Percentage per day.
Days—		Per cent.
20 to 25	0·2824	28·24
40 to 45	0·1350	13·50
60 to 65	0·0860	8·60
80 to 85	0·0620	6·20
100 to 105	0·0479	4·79
120 to 125	0·0387	3·87
140 to 145	0·0323	3·23

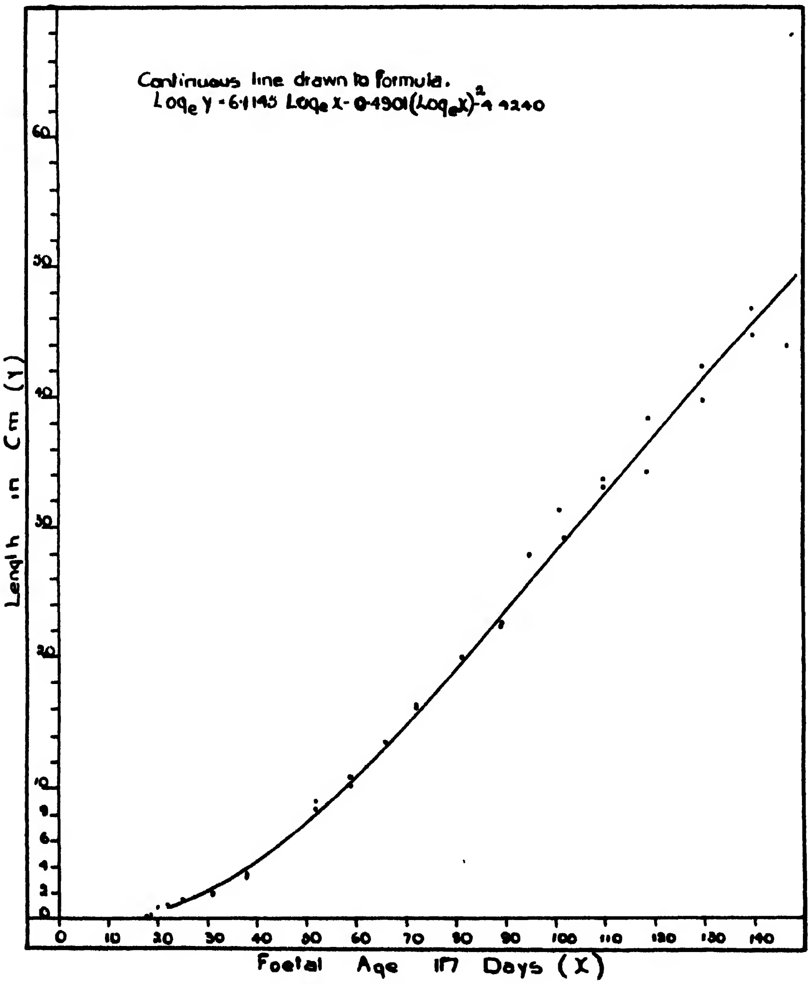
The table indicates clearly that the percentage rates calculated by Minot (1908) and others, which run into several hundred *per cent.*, are excessively high and that the drop in the earlier stages is not nearly as exaggerated as these workers lead one to expect. The rates are comparable with those calculated by Brody (1927*b*) for the rat (53 per cent.), the guinea-pig (10 per cent. to 4·6 per cent.) and the human being (8 per cent. to 1·25 per cent.). It is also

interesting to note that Brody (1927*b*) calculates the percentage daily growth-rate of the sheep during the first few weeks of post-natal life to be approximately 2 per cent., which is only slightly lower than that given above for the 140-145 day period, thus lending weight to the contention that the incident of birth is not accompanied by any drastic change in the growth-rate.

2. *Growth in Length.*

In view of the conflicting opinions as to the best method of determining the length of foetuses, and the fact that these opinions all rest upon a theoretical basis, it has been decided to consider here a few of the most usual methods, and from the results of statistical tests to conclude which are the most useful as a guide to foetal linear development. All the dimensions considered are described in Chapter 3.

Fig. 25.—Growth of Straight Crown-rump Length.



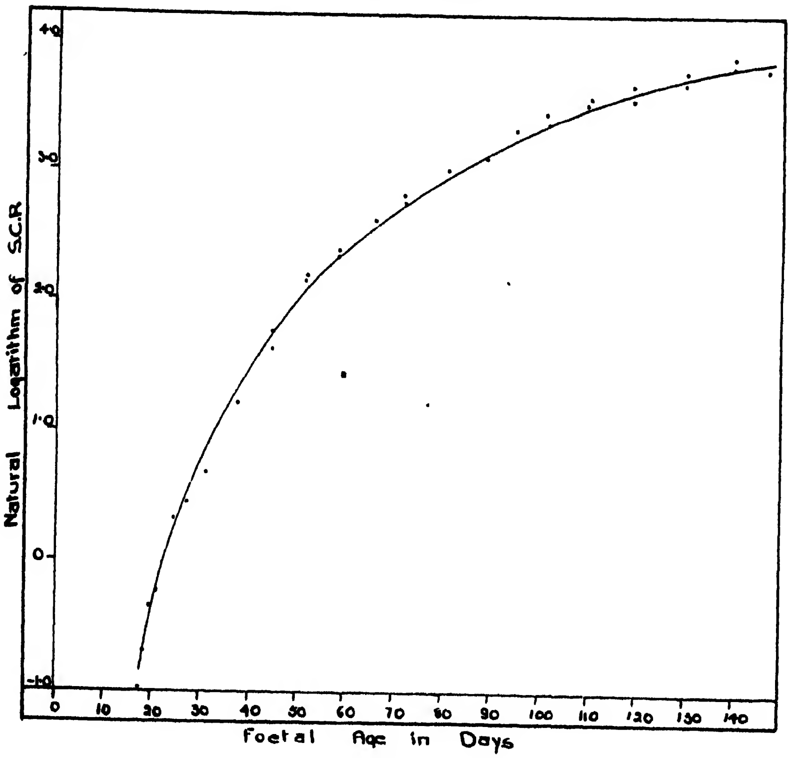
The data for *straight crown-rump length* are best fitted by a curve of the second degree having the formula:—

$$\text{Log}_e\ \text{C.R.}(s) = 6\cdot1145\ \text{Log}_e\ \text{Age} - 0\cdot4901\ (\text{Log}_e\ \text{Age})^2 - 14\cdot4240.$$

This curve is definitely significant at the 1 per cent. level of probability, while the coefficient of variation is 7·51 per cent. In Fig. 25 it will be seen that the data are well distributed about this line. Further, it is noticed that the second inflection is not very distinct and that the curve does not resemble an elongated "S" as stated by Curson and Malan (1935). However, it approximates fairly closely to Draper's (1920) guinea-pig curve.

In Fig. 26 is presented the instantaneous growth-rate curve of straight crown-rump length. Again the curve is smooth, without any abrupt breaks and lies concave to the axis of the graph. In general shape it conforms closely to that of the weight data.

Fig. 26.—Curve for Instantaneous Growth-rate of Straight Crown-rump Length.



Details of the percentage growth-rate of this dimension are tabulated in Table 28. It is noticed not only that this rate is lower at each stage than that of the weight, but also that the decrease in the rate towards the end of foetal life is much more exaggerated here. As a result of this greater "slowing down" in linear growth

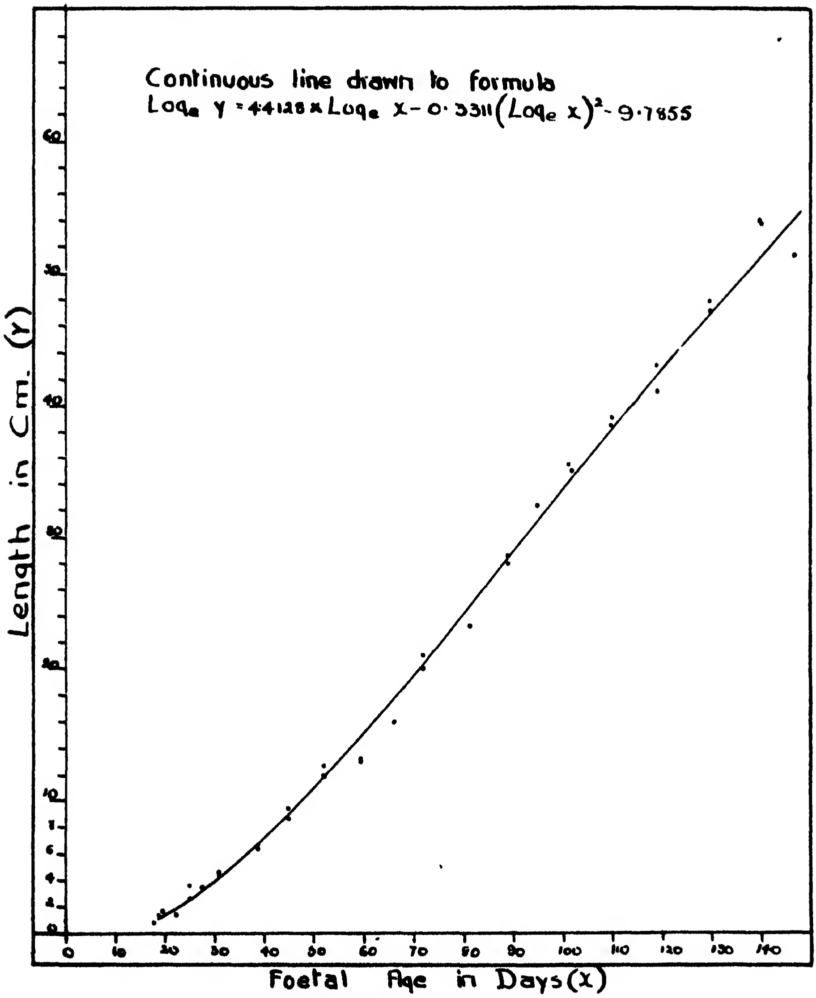
the second inflection of the growth curve has been appreciated by workers who have failed to detect its existence in connection with weight data.

TABLE 28.

Growth Rates.—C.R. (s) Per Cent. Per Day.

Age.	Value of K.	Percentage per day.
Days—		Per cent.
20 to 25	·1370	13·70
40 to 45	·0575	5·75
60 to 65	·0330	3·30
80 to 85	·0217	2·17
100 to 105	·0154	1·54
120 to 125	·0114	1·14
140 to 145	·0088	0·88

Fig. 27.—Growth of Curved Crown-rump Length.

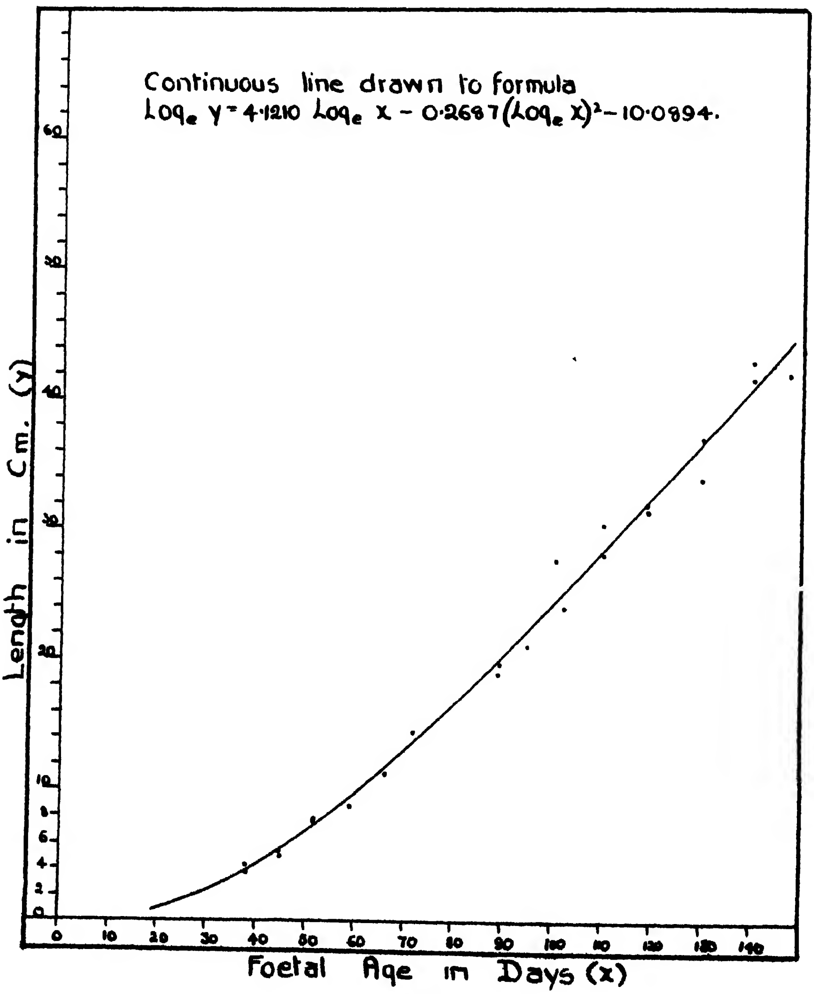


The second dimension to be considered is Curson and Malan's (1936*a*) *curved crown-rump length.* The best-fitting curve is a parabola similar to those already considered. The formula is:—

$$\text{Log}_e \text{ C.R.}(c) = 4 \cdot 4128 \text{ Log}_e \text{ Age} - 0 \cdot 3311 (\text{Log}_e \text{ Age})^2 - 9 \cdot 7855.$$

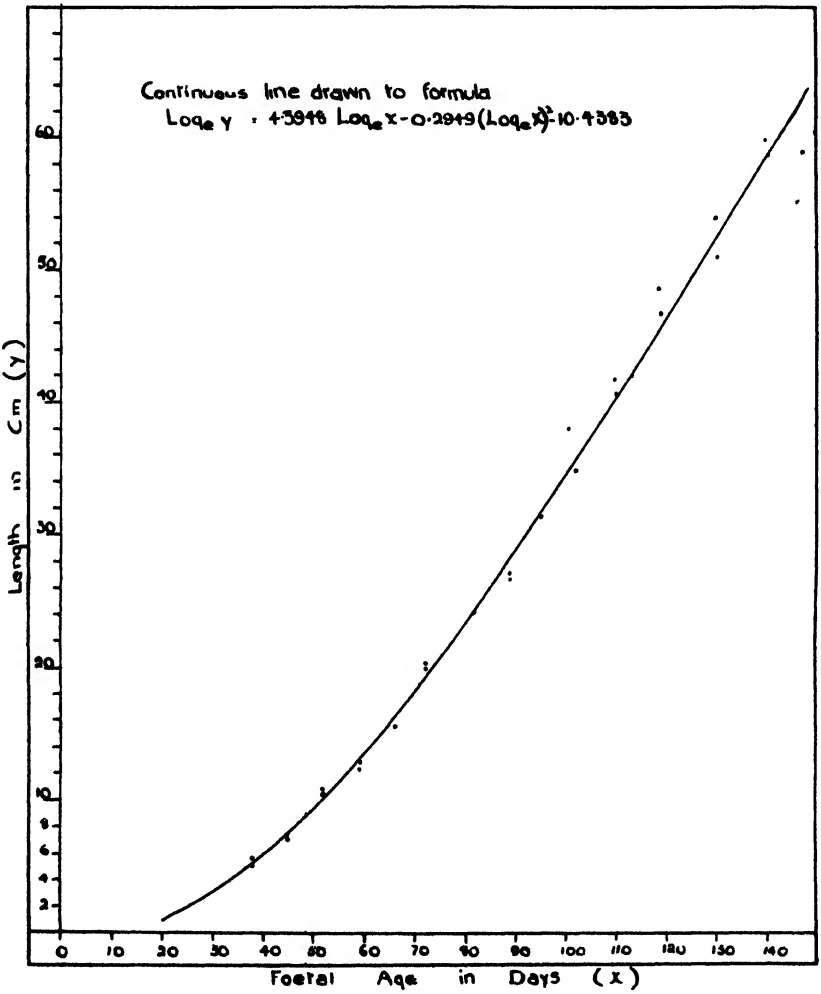
Although the significance is marked, the coefficient of variation is 10·34 per cent. In Fig. 27 the data are less evenly distributed than those of the straight crown-rump length. Thus one concludes that in spite of the theoretical advantages of the curved over the straight line, the latter, when measured according to a set plan, is the less variable and hence the more useful of the two. This had already been indicated in the work of Malan and Curson (1936*a*) in whose weight-length diagrams the curved measurement gave a less satisfactory " fit " than that obtained with the straight line dimension.

Fig. 28.—Growth of Length of Back-line.



Undoubtedly a fair proportion of the variation of this measurement is directly attributable to the difficulty of taking precise readings when one of the landmarks is as vague as the " root of the tail ". Apart from this technical difficulty there is the fact that this dimension is affected not only by growth in the direction of the long axis of the body, but also by growth at right angles to this axis, i.e. in the length of the head. It is difficult to see how such a " composite " line can be expected to represent satisfactorily the linear enlargement of the body.

Fig. 29.—Growth of Length in the Vertebral Column.



In order to avoid the above criticism the head portion of the curved line has been omitted in the measurement of the *back-line*. The data obtained are plotted in Fig. 28, in which the continuous line has been drawn to the formula:—

$$\text{Log}_e \text{ Black-line} = 4 \cdot 1210 \text{ Log}_e \text{ Age} - 0 \cdot 2687 (\text{Log}_e \text{ Age})^2 - 10 \cdot 0894.$$

This formula is highly significant even at the 1 per cent. level of probability. In this instance the coefficient of variation is 4·93 per cent., which must be regarded as extremely satisfactory. However, in the determination of this dimension the root of the tail is again the caudal landmark. Consequently it is not surprising to find that by measuring the *length of the entire vertebral column* (i.e. the foregoing length plus the tail) the coefficient of variation can be reduced to 3·8 per cent. The formula for this curve, shown in Fig. 29, is:—

$$\text{Log}_e \text{ V.C.} = 4\cdot3948 \text{ Log}_e \text{ Age} - 0\cdot2949 (\text{Log}_e \text{ Age})^2 - 10\cdot4383.$$

The tests for significance yield positive results at the level of $P = \cdot01$.

This dimension differs from Mall's (1910) "vertebral column length" for the human being in that in the latter the length of the tail is disregarded. In the sheep foetus the tail is relatively long, hence, perhaps, its greater importance. However, it appears that the slight superiority of the vertebral column length over the back-line is the result of greater accuracy in the measurement of the former, due entirely to the fact that in this case both landmarks are specific.

In Table 29 are tabulated details of the percentage growth-rate of the vertebral column length. The corresponding figures for the back-line resemble these very closely.

TABLE 29.

V.C. Length. Per Cent. Growth-Rate.

Days.	Value of K.	Percentage per day.
Days—		Per cent.
20 to 25	0·1094	10·94
40 to 45	0·0496	4·96
60 to 65	0·0304	3·04
80 to 85	0·0212	2·12
100 to 105	0·0159	1·59
120 to 125	0·0126	1·26
140 to 145	0·0102	1·02

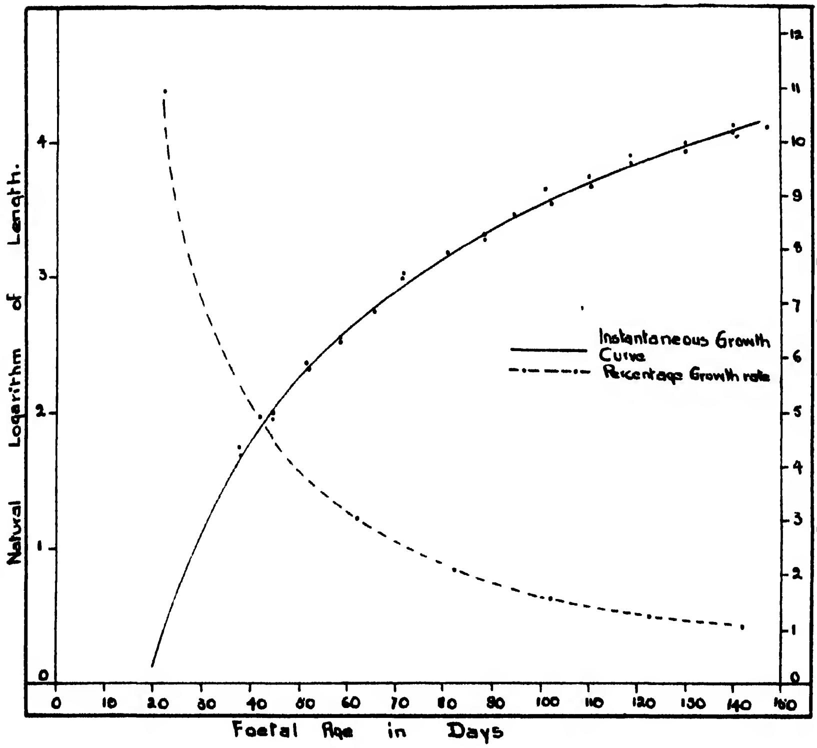
In Fig. 30 is presented the instantaneous growth-rate curve of this dimension. The actual observations follow very closely the smooth curve drawn from calculated lengths. Again there is no suggestion whatsoever of straight segments with sudden "breaks". The shape of the curve is similar to that obtained with the weight data; so too is the graph of average percentage growth-rates which is also plotted in Fig. 30.

It is found that the trend of growth in all four of these linear dimensions is best represented by curves of the second degree. In no instance is the second inflection at all distinct. There is a fair

degree of similarity in the average percentage growth-rates of these dimensions, and at each period they are all well below the rate of growth in weight.

Considering the coefficients of variation of these lines it is found that the vertebral column length and the back-line are the least variable, and hence the most useful dimensions. The straight crown-rump length is slightly inferior to them, but it is especially useful in connection with foetuses younger than 35 days, in which the first two dimensions cannot be determined with any degree of accuracy.

Fig. 30.—Curves for Instantaneous Growth-rate and Average Percentage Rate of Growth of Length of Vertebral Column.



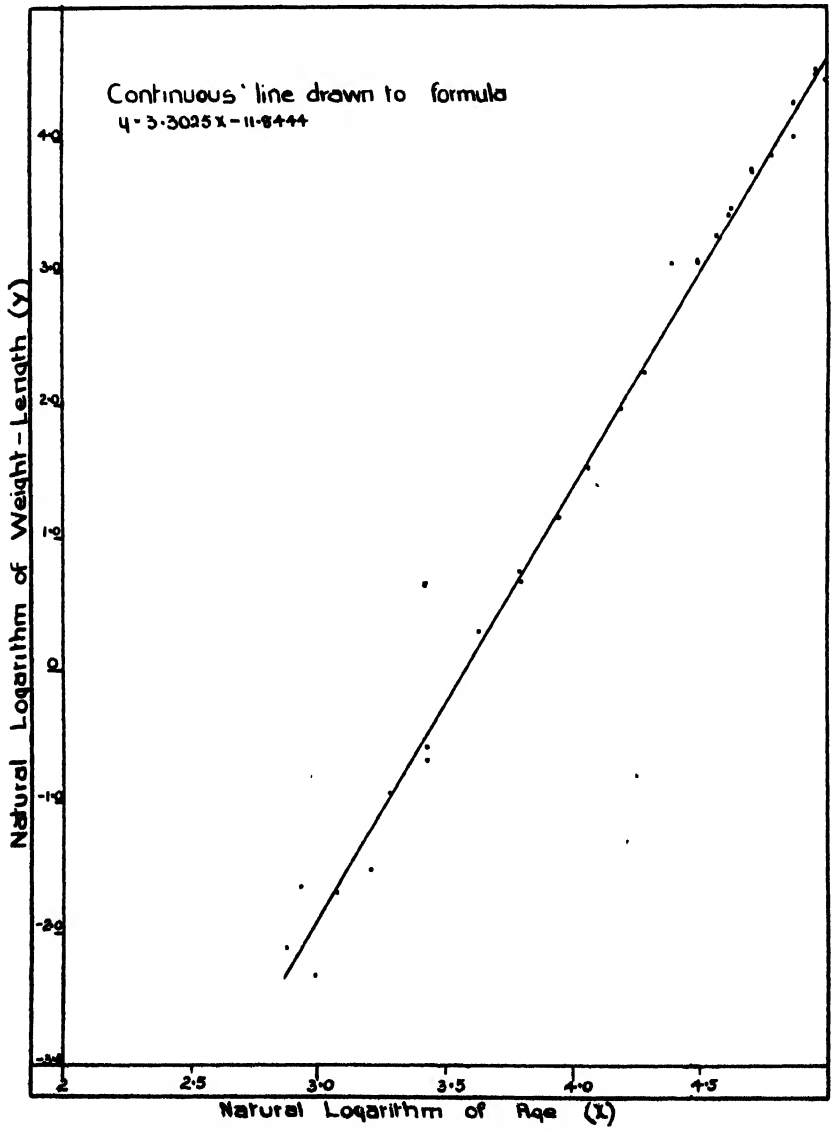
The curved crown-rump length is definitely the least useful of the measurements considered and, remembering the other criticisms of this line, may be discarded.

It is to be noted that the coefficient of variation of this discarded line is only half that of the weight-curve, while regarding vertebral column length this fraction is reduced to one-fifth. These findings are contrary to the assertion of Curson and Malan (1936*a*) that "weight and length are equally variable". Hammond's (1927) statement that "weight is more variable than length" is applicable with equal force to the foetal, as well as the post-natal period of animal life.

3. "*Weight-Length" Ratio.*"

In studying the Weight-Age and Length-Age curves just described, it is found that when the weight of a particular foetus lies above the trend line, the point representing the length of that foetus also tends to fall above the smooth curve. This suggests that there may be a distinct correlation between these two characteristics and that it may be profitable to calculate for each foetus the weight-length ratio, and to study the trend of this ratio with a view to determining whether it would not prove less variable than either of the two dimensions from which it is calculated.

Fig. 31.—Relation between Weight-Length Ratio and Age.



Although it has been proved that the length of the vertebral column is the least variable of the length measurements, it is felt that, on account of its total disregard of the head, this dimension is not the most suitable for the purpose of calculating a weight-length index. Consequently, the straight crown-rump length is preferred, and in each instance this measurement in centimetres is divided into the weight in grams.

In Fig. 31 it is shown that the plotting of the natural logarithms of these indices against those of the corresponding ages results in a straight-line distribution. The continuous line in the figure is drawn to the formula:—

$$\mathrm{Log}_e \text{ W. L. Ratio} = 3{\cdot}3025 \ \mathrm{Log}_e \text{ Age} - 11{\cdot}8444,$$

which is highly significant, but has a coefficient of variation of 17·3 per cent. It is quite evident that the computation of this index will not serve any useful purpose.

Figure 32 is inserted mainly to indicate that the formula mentioned does actually represent the true trend of the data and that the large coefficient of variation is due entirely to occasional marked deviations from this general line.

4. *Regional Growth.*

Growth of the body as a whole is merely the sum total of growth of all its component parts. If the rates of growth of all these parts were identical the body would increase in size without in any way changing its shape or its proportions. However, it is well known that this is not the case, and that growth and moulding of shape proceed hand in hand. Regarding the sheep foetus this is well illustrated in an admirable chart presented by Curson and Malan (1935). Here outline sketches of a series of foetuses have been constructed to such scales that in every instance the height at the withers (i.e. the vertical distance from withers to tip of toe) is identical. With their background of squared paper these illustrations allow of a full appreciation of the changes in proportion which accompany the growth in size of the foetus. It is surmised that such changes must be attributable to differential regional growth-rates.

In an effort to test this hypothesis in a statistical manner, and to obtain some idea of the mode and sequence of operation of these differential rates, a number of linear measurements in different regions of the body have been recorded. When the trends of these data are studied it is found that in each instance the best fitting curve is a parabola with the general formula:—

$$\mathrm{Log}_e \text{ Dimension} = a + d \ \mathrm{Log}_e \text{ Age} + c \ (\mathrm{Log}_e \text{ Age})^2,$$

in which a, b and c represent constants which differ for each dimension considered. The values of these constants are tabulated in Table 30, in which are also indicated the coefficients of variation and the results of the tests for significance. Regarding the latter X indicates significance at the 5 per cent., and X X at the 1 per cent. level of probability.

Fig. 32.—Change of Weight-Length Ratio with Foetal Age.

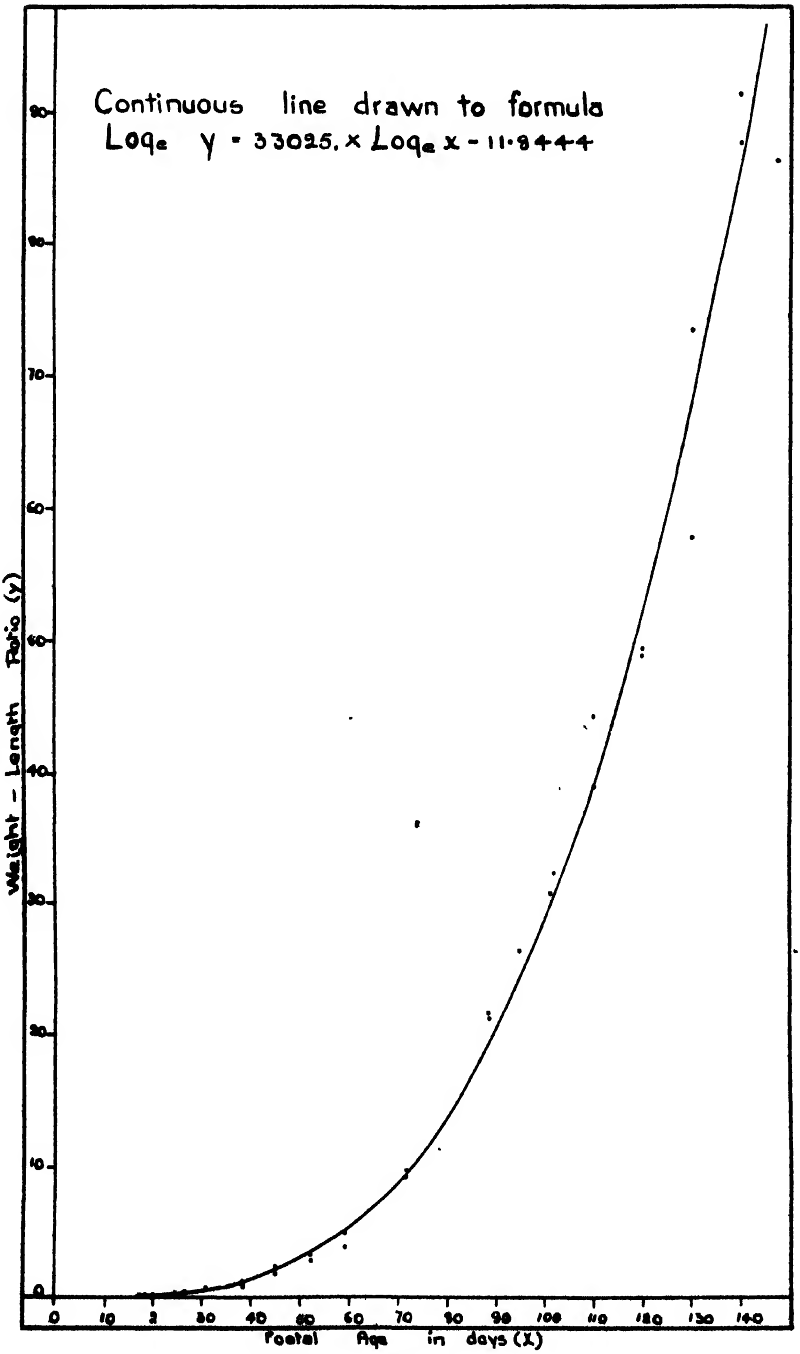
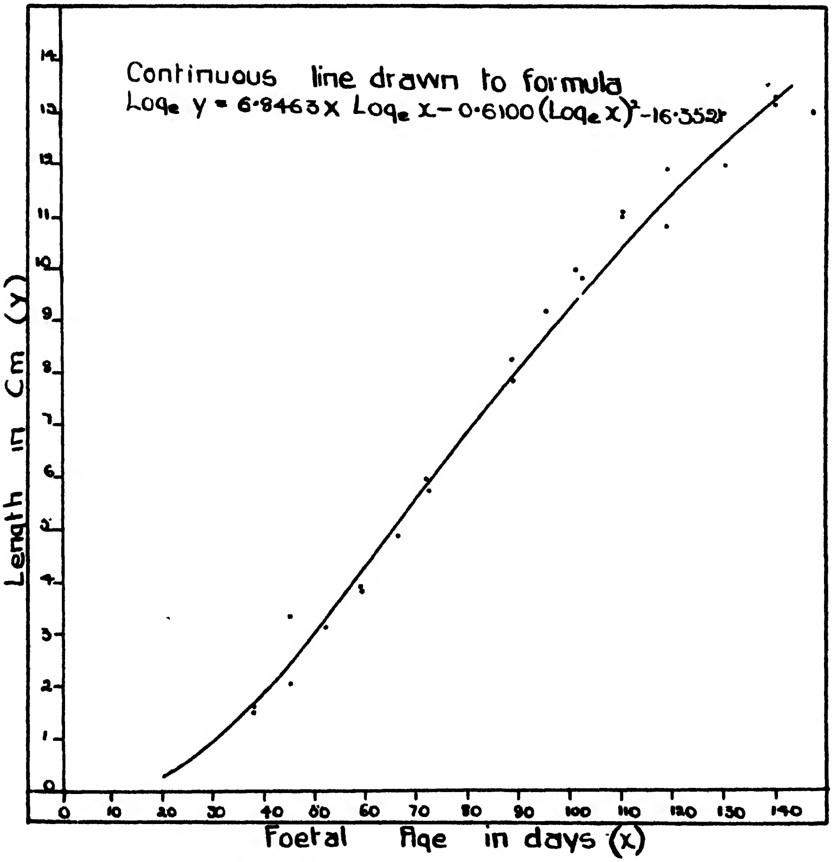


TABLE 30.

Constants for Formulae for Growth of Regions.

Dimension.	a.	b.	c.	Coeff. Var.	Significance.
Length of head....	-16·3521	+6·8463	−0·6100	8·76	XX
Length of neck....	+ 3·6166	−2·2939	+0·4114	16·71	—
Length of trunk...	-18·9550	+7·8031	−0·6635	4·07	XX
Length of tail.....	−13·4313	+5·0861	−0·3609	9·75	XX
Length of f. limb..	−15·3832	+5·9662	−0·4289	4·72	XX
Length of h. limb..	−17·5845	+6·8646	-0·5128	4·88	XX

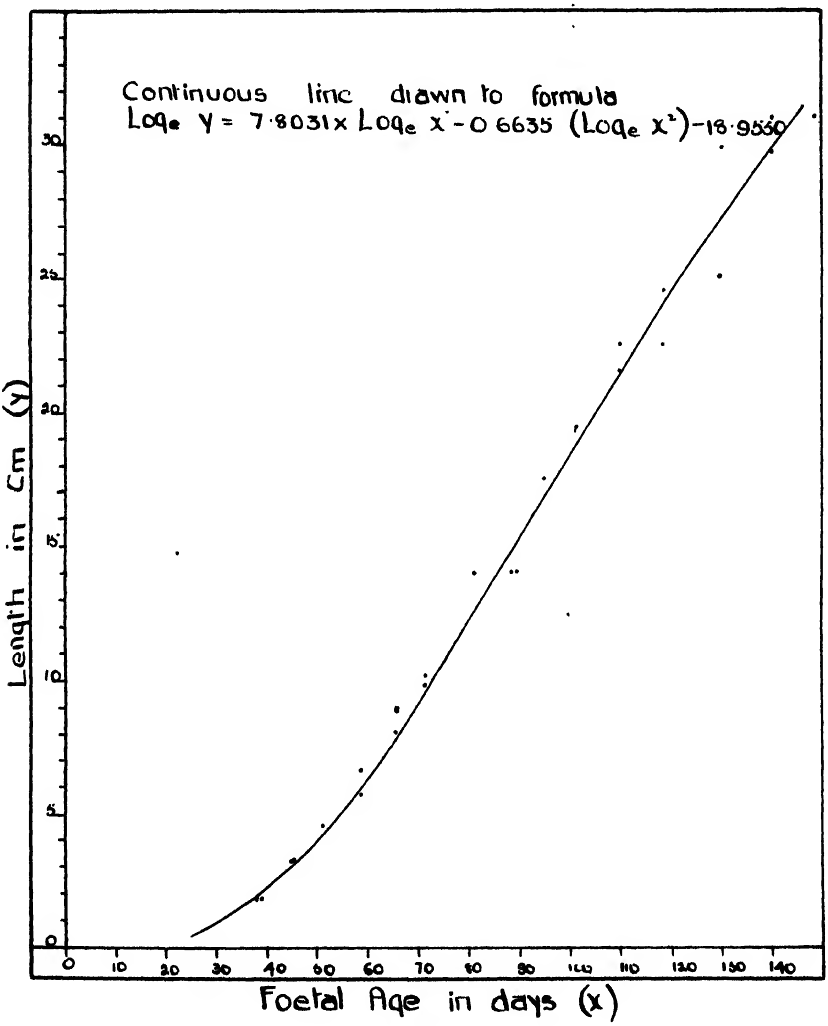
Fig. 33.—Length of Head.



In all but one dimension the values of the constants are strikingly similar, the coefficients of variation are well below 10 per cent., and significance is marked. The curves drawn to these formulae are presented in Figs. 33 to 37. Most of these curves resemble closely those previously encountered. In the case of the

head and, to a lesser extent, the trunk, the second inflection is more plainly visible than in any of the previous figures. There is no doubt but that in their growth all these regions follow trends similar to that of the body as a whole.

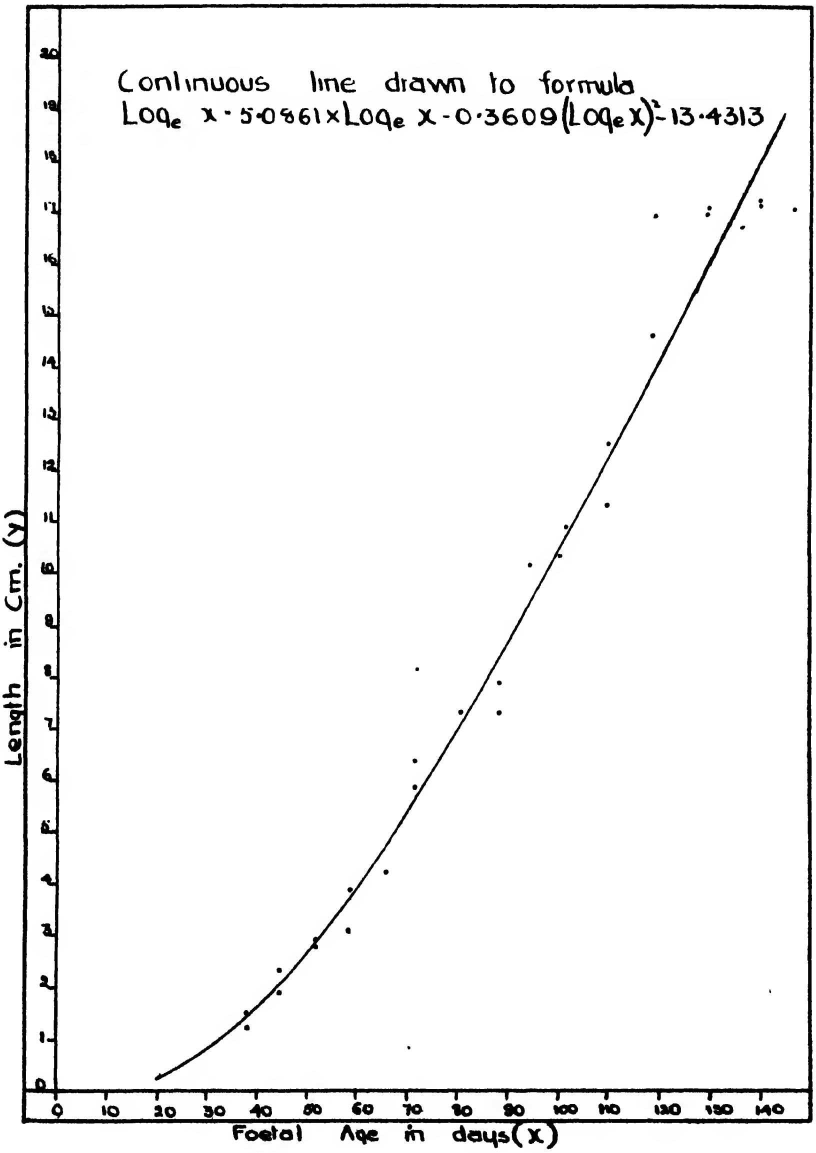
Fig. 34.—Length of Trunk.



The striking exception is the neck. Here the figures are greatly at variance with those of the other dimensions. The signs of the constants have also been reversed, thus the inflections of the parabola constructed from this formula will be inverted, i.e. the first will be concave, and the second convex to the axis of the figure. Had this curve been significant there would have arisen the difficulty of explaining why of all these regions, the neck should follow such an unique growth-trend. However, it is found that the coefficient

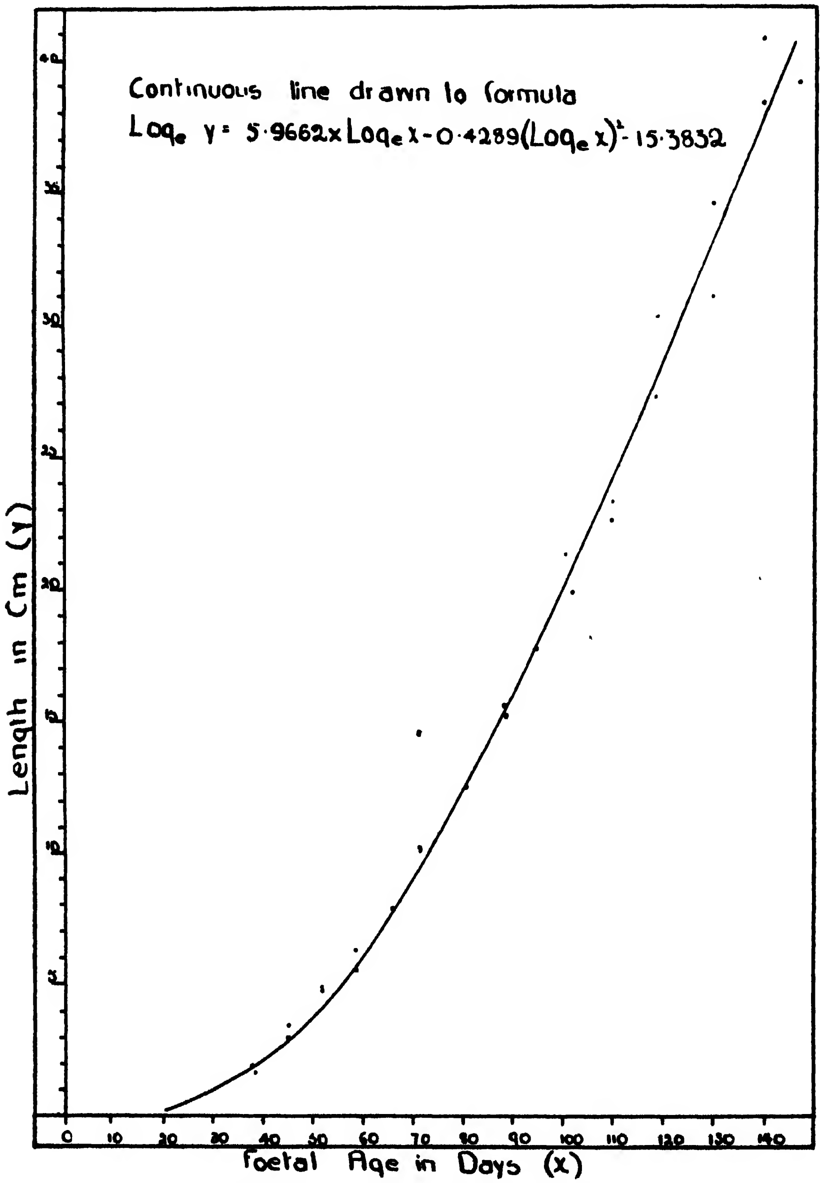
of variation is over 16 per cent. and that both factors b_1 and b_2 (see Appendix B) are totally insignificant. This indicates that the degree of variation in the original data is such as to make it impossible to construct a curve which will represent accurately the trend of these data. It must be remembered that the length of the neck was obtained by deducting from the back-line the length of the trunk. From the above results it is concluded that this is not

Fig. 35.—Length of Tail.



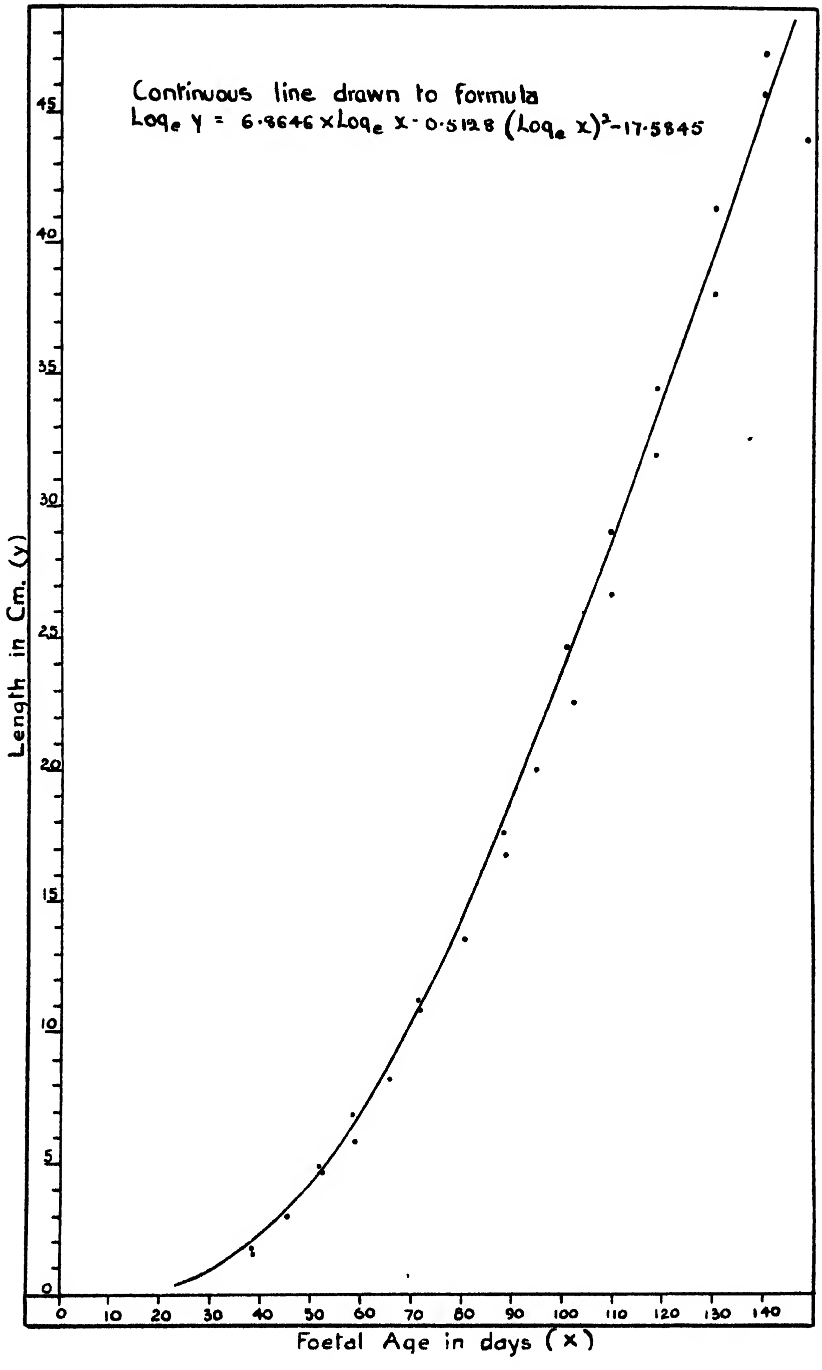
a satisfactory method of estimation. Consequently this apparently conflicting evidence is disregarded, and so far the hypothesis that the growth-trends of all regions follow those of the entire body remains unassailed.

Fig. 36.—Length of Forelimb.



Having found that there is no qualitative difference in the growth processes of the several regions under consideration, we may conclude that the differential nature must consist in the percentage growth-rates, i.e. it must be quantitative. In Table 31 are presented

Fig. 37.—Length of Hindlimb.



details of the average percentage growth-rates of these dimensions, calculated over periods of five days at 20 day intervals. It must here be stressed that the values for the first period (between the ages of 20 and 25 days) are purely theoretical, having been obtained from the formulae by extrapolation. Although the formula may have been proved to hold within the range 38 days to 147 days, this does not necessarily mean that it will furnish correct values when its use is extended beyond either limit of the range. However, these values may serve a purpose provided one does not lose sight of their purely theoretical nature.

TABLE 31.

Percentage Growth-Rates of Regions.

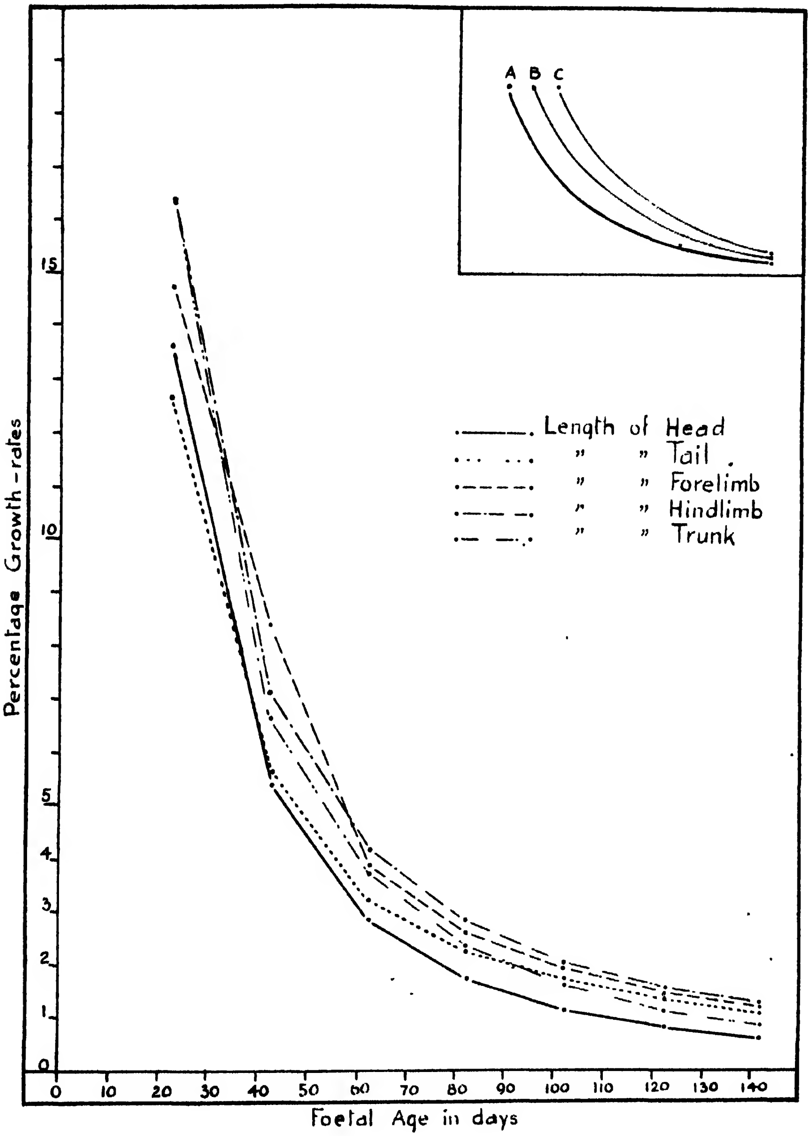
Age.	Growth-Rates.—Percentage per Day.				
	Head.	Trunk.	Tail.	Forelimb.	Hindlimb.
Days—					
20 to 25......	13·64	16·43	12·69	14·73	16·42
40 to 45......	5·36	6·67	5·61	8·48	7·12
60 to 65......	2·89	3·71	3·37	3·88	4·20
80 to 85......	1·78	2·36	2·31	2·65	2·84
100 to 105......	1·17	1·62	1·70	1·93	2·07
120 to 125......	0·80	1·16	1·32	1·50	1·58
140 to 145......	0·56	0·86	1·05	1·20	1·25

In Fig. 38 these percentage rates are all plotted against age, the midpoint of the five day period over which the rate has been calculated being taken for the abscissal reading.

It becomes apparent that in each instance the rate of growth is highest at the earliest stage and then decreases, at first rapidly, but later more gradually. Further, in the earliest period all the rates are roughly of the same order. The lowest rate (tail) is 77 per cent. of the highest one. By the end of prenatal life these rates have all undergone pronounced reduction. At this time the differences between them are much greater, the lowest rate (head) constituting but 45 per cent. of the highest rate (hindlimb). The most cranial portion of the body has the lowest rate, while the highest rate is encountered in the hindlimb—the part most remote from the head.

If the crown of the head is regarded as the starting point of growth, it is found in Table 31 that the more remote the region is from this point the higher is its growth-rate at the end of the prenatal period, i.e. the less has been the retardation to its initial rate. Also, the more distant the region the later (chronologically) will be the moment of inception of growth in that region. From these facts it is concluded that there is a direct relation between the extent of retardation of the growth-rate and the time that has elapsed since the onset of growth in that particular part.

Fig. 38.—Percentage Growth-rates of different regions.



On this basis it is incorrect to look upon the rates tabulated in the first line of Table 31 as representative of the initial growth-rates of the regions under consideration. Obviously in the head region there will already have been a fair degree of retardation, while in the hindlimb this will be at a minimum. To arrive at the true initial or commencing rates of these regions it would be necessary to increase the rates of the first period by amounts varying in decreasing order from the head to the hindlimb. This would have the effect of eliminating the variations encountered in the first line

of the table, with the result that all the initial rates would be more or less identical. The rate for the trunk is slightly confusing, yet it must be remembered that this region is large and that while some of its components are in close proximity to the head, others are definitely remote from it. In the latter parts growth will commence some time later than in the former.* This initiation of "new" growth will tend to minimise the extent of the retardation which will already have set in anteriorly. Thus the "slowing down" of the rate for the trunk will not be marked in the earliest stage, placing it in the anomalous position seen in the table. Later in prenatal life this anomaly disappears and the values for its rate of growth fall correctly between those of the head and the tail.

It appears that growth is initiated in the head region and that it spreads wave-like caudally and towards the extremities; in each region the initial or potential growth-rate is the same; from the moment of onset of growth an inhibiting force is at work reducing the rate in a manner proportional to the lapse of time since the commencement of growth in the region concerned. The tendency of this force is to reduce the rate to zero, but in no instance is that level reached during prenatal life. This is only to be expected, since growth ceases only after attainment of full adult size. However, the earlier growth has commenced in any region, the nearer will the rate at the end of prenatal life be on this ultimate zero.

All these points are well illustrated in Fig. 38, the last half of which is very regularly arranged. In the first part of the figure this regularity is slightly disturbed, some of the lines crossing each other. This may be indicative of some inherent differences in the modes of retardation in the various organs. One may be tempted to assume that this is the case and then, by way of explanation, recourse may be had to the expedient of linking up certain points on the curve with certain concurrent events in the development of the foetus. Such reasoning, although not infrequently resorted to, does not appear to be justified. Moreover, having regard to the nature of the data employed in this study, one hesitates to attach great significance to the slight irregularities observed. Indeed, it is suggested that the true state of affairs may be as idealized in the inset in Fig. 38, where points A, B and C represent both the commencing times and rates of growth of three dimensions placed in order according to their antero-posterior sequence.

As a result of the findings recorded in this section it is concluded that during the course of prenatal life the proportions of the body change as the result of differential time rates of retardation of growth in the various regions of the body. Thus the more anterior regions begin growing at an earlier age and obtain a "start" on those parts situated further caudally. As the rates of growth of the former regions undergo retardation, the relatively higher

* Obviously the same applies to any other region, but naturally the effects will not be so marked in a relatively small region as they are in a fairly extensive and elongated region.

rates of the latter regions (consequent upon their more recent entry into the growth process) enable these parts to increase their relative size and thus eliminate the earlier disproportion.

All the dimensions considered above are measurements in the direction of the long axes of the regions concerned. Merely to see whether the trends obtained here are applicable to dimensions measured in other directions, the width of the head and the circumference of the thorax (heart-girth) have been included in this study. The former is measured at right angles to the length of the head, while the latter is a circumferential measurement in a vertical plane. In both cases curves of the second degree are found to represent the trends of the data. The formulae are:—

Width of Head.

$$\mathrm{Log}_e \text{ Width} = 6{\cdot}5252 \,\mathrm{Log}_e \text{ Age} - 0{\cdot}5815 \,(\mathrm{Log}_e \text{ Age})^2 - 16{\cdot}1182.$$

This is definitely significant at $P = 0{\cdot}01$ and the coefficient of variation is 4·75 per cent. This curve is illustrated in Fig. 39.

Heart-Girth.

$$\mathrm{Log}_e \text{ H.G.} = 4{\cdot}9768 \,\mathrm{Log}_e \text{ Age} - 0{\cdot}3760 \,(\mathrm{Log}_e \text{ Age})^2 - 11{\cdot}8658.$$

Again this is significant at the 1 per cent. level of probability, and the coefficient of variation is 5·48 per cent. Graphic representation is given in Fig. 40.

It will be noticed that the first curve (width of head) resembles closely that of the length of head. The second inflection is again distinctly visible. The second curve is of the same shape as those for the trunk and the forelimb.

Thus it is established that dimensions other than those determined in the plane of the long axis of a region also follow the growth-trend of the whole body.

TABLE 32.

Percentage Growth-Rates.

Age in Days.	Rates—Percentage per Day.	
	Width of Head.	Heart-Girth.
20 to 25	13·00	11·79
40 to 45	5·10	5·09
60 to 65	2·75	2·99
80 to 85	1·69	2·01
100 to 105	1·11	1·46
120 to 125	0·72	1·11
140 to 145	0·53	0·88

The percentage growth-rates of these two dimensions are tabulated in Table 32. These, it will be noticed, are comparable with the rates of other dimensions of the same regions. In the

case of the head it is seen that length and width have roughly similar rates, but that of the latter is always somewhat lower. In the earlier stages the rate for the heart-girth is slightly confusing, but it soon assumes a close similarity to the rate of linear growth of the trunk.

Fig. 39.—Width of Head.

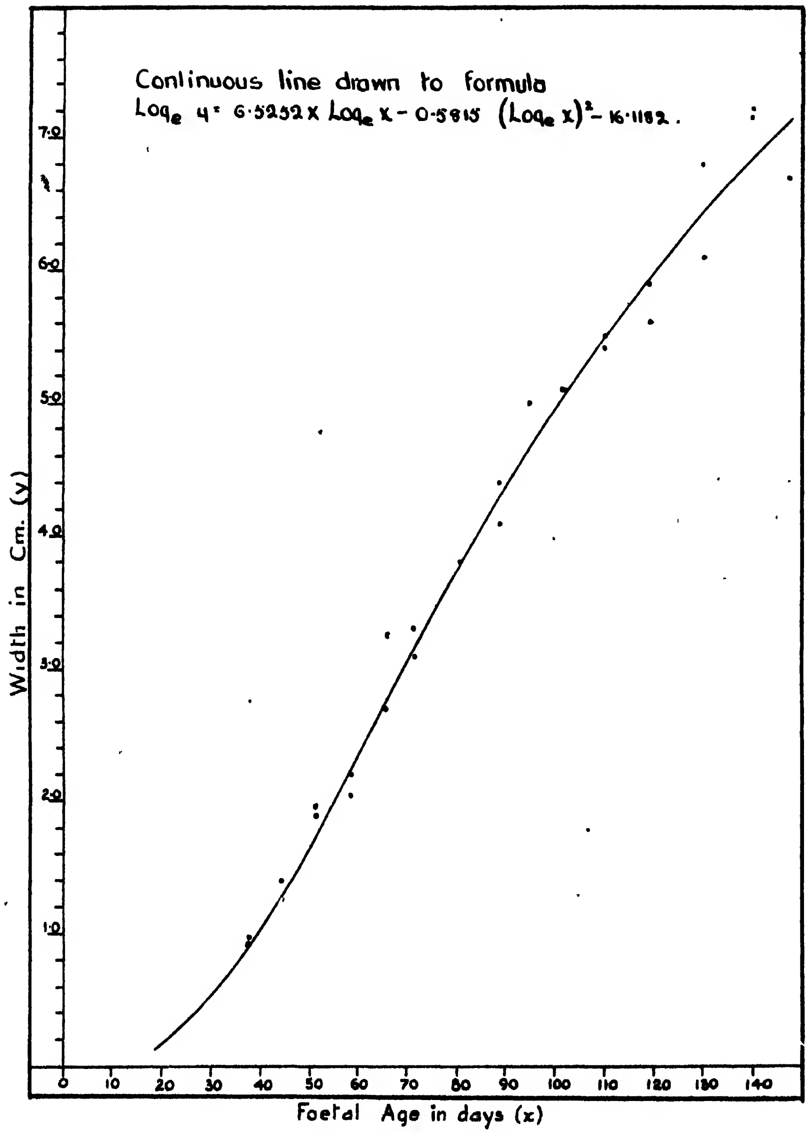
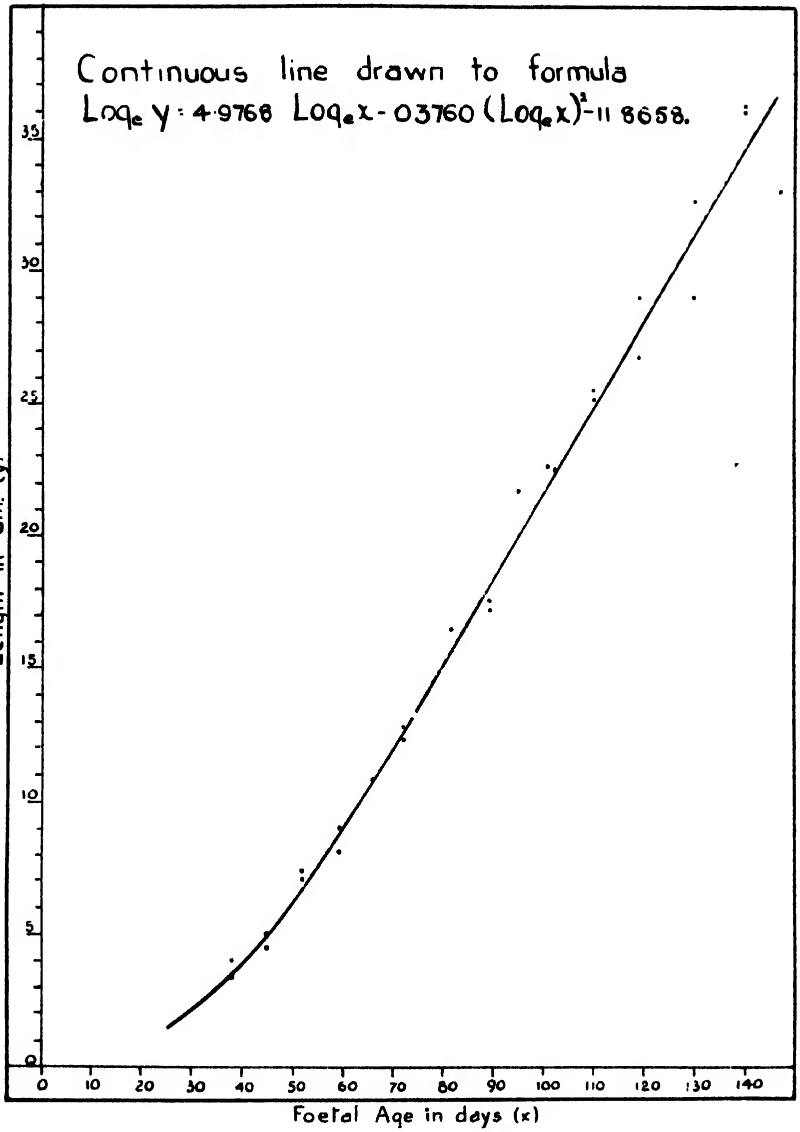


Fig. 40.—Length of Heart-Girth.



5. *Correlation Between Dimensions.*

In the previous two sections it has been found that both the actual observations and the fitted curves of all the dimensions considered exhibit striking similarities. This suggests that between any two of these dimensions there may be some definite relationship. For the purpose of testing the accuracy of this surmise the coefficients of correlation between these dimensions are determined. The method employed is that described by Fisher (1936) for the estimation of " correlation between series ".

Since measurements of these variables have extended over a period of growth it is only to be expected that between them there will be a high positive correlation. However, this "total correlation" may be split into two components, one of which, the "trend correlation", measures the relationship between points on the fitted curves of the various dimensions, or, in other words, the correlation between values of the dimensions calculated from their regression formulae. The other component measures the correlation of the deviations of the actual observations from one trend-line, with the deviations of the corresponding observations from any other trend-line. This "residual correlation" is the important one in this work for in its computation the growth-trend has been eliminated, hence it indicates exactly how a deviation of one dimension from the "normal" or "expected" value is likely to be reflected in any other dimension.

Table 33 has been compiled in such a manner that all three correlations between any two variables may be read at a glance. It will be noticed that the portion falling below the diagonal row of blank spaces is an exact repetition of the upper half. Through its inclusion it is possible to read in one straight line, either horizontally or vertically, correlations of a certain dimension with all the remaining variables. Otherwise it would have been necessary to follow a vertical column into the blank space and then to continue along the horizontal column.

In considering the figures presented in the table each of the three types of correlation will be treated separately.

Total Correlation.

It is seen that without exception these figures are highly significant at the level $P=0\cdot01$. Thus there is a very high, positive, direct correlation between all the dimensions under consideration. As explained above, this is only to be expected.

Trend Correlation.

Again there is not a single figure which is not significant at at least the 5 per cent. level of probability. However, it is possible to classify the variables in two groups; in the first are length of head, width of head and length of trunk, while the remainder constitute the second group. The correlation between any two variables of the same group is significant at $P=0\cdot01$, whereas correlations between dimensions of opposite groups are significant only at $P=0\cdot05$. These results could have been anticipated from a study of the continuous curves in Figs. 34-40. Those representing the trends of the dimensions of the first group show much similarity, and in all of them the second inflection is distinct. The remaining curves, while differing from the above, bear a striking resemblance to one another.

TALLE 33.

Correlation Coefficients.

		Length V.C.	Length Trunk.	Length Head.	Length Tail.	Length Forelimb.	Length Hindlimb.	Width Head.	Heart Girth.
Length of V.C.	1	—	0·9998	0·9966	1·0000	1·0000	1·0000	0·9968	1·0000
	2	—	*0·2111*	*0·1393*	*0·5295*	*0·4416*	*0·4893*	*0·3127*	*0·5887*
	3	—	0·9966	0·9880	0·9956	0·9984	0·9985	0·9937	0·9978
Length of Trunk	1	0·9998	—	0·9999	0·9989	0·9991	0·9994	1·0000	0·9996
	2	*0·2111*	—	*0·3200*	*0·0010*	*0·3132*	*0·4010*	*0·3573*	*0·3586*
	3	0·9966	—	0·9925	0·9915	0·9971	0·9978	0·9317	0·9968
Length of Head	1	0·9966	0·9999	—	0·9971	0·9974	0·9979	1·0000	0·9982
	2	*0·1393*	*0·3200*	—	*0·3987*	*0·3625*	*0·3088*	*0·4943*	*0·3033*
	3	0·9980	0·9925	—	0·9885	0·9903	0·9904	0·9945	0·9901
Length of Tail	1	1·0000	0·9989	0·9971	—	1·0000	1·0000	0·9973	1·0000
	2	*0·5295*	*0·0010*	*0·3987*	—	*0·2946*	*0·1852*	*0·1855*	*0·3010*
	3	0·9956	0·9915	0·9885	—	0·9941	0·9934	0·9899	0·9935
Length of Forelimb	1	1·0000	0·9991	0·9974	1·0000	—	1·0000	0·9975	1·0000
	2	*0·4416*	*0·3132*	*0·3625*	*0·2946*	—	*0·8166*	*0·5979*	*0·3833*
	3	0·9984	0·9971	0·9903	0·9941	—	0·9995	0·9957	0·9972
Length of Hindlimb	1	1·0000	0·9994	0·9979	1·0000	1·0000	—	0·9981	1·0000
	2	*0·4893*	*0·4010*	*0·3088*	*0·1852*	*0·8166*	—	*0·4803*	*0·3262*
	3	0·9985	0·9978	0·9904	0·9934	0·9995	—	0·9957	0·9971
Width of Head	1	0·9968	1·0000	1·0000	0·9973	0·9975	0·9981	—	0·9983
	2	*0·3127*	*0·3573*	*0·4943*	*0·1855*	*0·5979*	*0·4803*	—	*0·5900*
	3	0·9937	0·9317	0·9945	0·9899	0·9957	0·9957	—	0·9960
Heart Girth	1	1·0000	0·9996	0·9982	1·0000	1·0000	1·0000	0·9983	—
	2	*0·5887*	*0·3586*	*0·3033*	*0·3010*	*0·3833*	*0·3262*	*0·5900*	—
	3	0·9978	0·9968	0·9901	0·9935	0·9972	0·9971	0·9960	—

NOTE.—

	P=0·05.	P=0·01.	
1. Trend Correlation	0·9969	0·9999	Values for Significance.
2. Residual Correlation	0·4227	0·5368	
3. Total Correlation	0·4227	0·5368	

It has been demonstrated that the distinct second inflection is associated with the dimensions of regions which develop early in prenatal life and consequently undergo the greatest degree of retardation of growth-rate. From the above findings one may conclude that "trend correlation" is greatest between dimensions of (anatomically) closely related regions, and lowest between dimensions of regions remote from each other.

Residual Correlation.

Immediately it is noticed that the majority of these correlation coefficients fail to attain significance even at the 5 per cent. level probability. Those that do reach this level may be considered individually:—

Length of forelimb—length of hindlimb.—The correlation between these two variables is the highest obtained. Upon inspection of Figs. 36 and 37 it is seen that there is much similarity not only in the trend-lines, but also in the distribution of the actual observations about these lines. Under these circumstances a high correlation is only to be expected. Moreover, it is fairly obvious why such a definite relationship should exist.

Length of head—width of head.—Again it is readily accepted that there should be a definite correlation between these variables. Indeed one is rather surprised not to encounter a higher value than 0·494. In Fig. 39 (width of head) it is noticed that the later points are widely spread, and that due to these marked variations in the actual data, the curve cannot be said to have an exceptionally good fit.* The large variations encountered may be inherent in the material or they may have been artificially exaggerated through the difficulty of making accurate measurements of this dimension.

Length of vertebral column—length of tail.—The high correlation present here is easily explained when it is remembered that the latter dimension is contained in the former. But for the difficulty of determining with accuracy the anterior extremity of the tail this correlation may well have been higher.

Length of vertebral column—heart-girth.

Length of vertebral column—length of forelimb.

Length of vertebral column—length of hindlimb.

* Nevertheless it has been proved that with the data available this curve gives the best "fit". What is meant here is that the "fit" of this "best-fitting" curve is not as good as is desirable in work of this nature.

In all these instances the relatively high correlations are understandable. It is quite acceptable that with an increase in the length of the foetus, there should be corresponding increases in the heart-girth and in the length of the two limbs. Thus a longer foetus has also a greater height at the withers and a larger circumference of the thorax.

Width of head—length of forelimb.
Width of head—length of hindlimb.
Width of head—heart-girth.

The definite correlations obtained here are somewhat surprising, especially in view of the fact that other seemingly more apparent correlations prove to be insignificant. However, it must be pointed out that in the method employed the accuracy of the residual correlation is dependent upon the "goodness of fit" of the trend-line. Already it has been indicated that in the case of width of head this fit is not as good as might be desired. It appears reasonable to conclude that this results in the generation of misleading correlations. In consequence of this suspicion attaching to these correlations it is not proposed to consider them further.

To sum up one concludes that between any two of the dimensions considered there is a very highly significant direct correlation. However, the common growth-trend is mainly responsible for this, and in most instances its elimination results in an insignificant residual correlation. The latter is truly significant in but a few cases, and then only when closely associated dimensions are coupled.

6. *External Appearance of the Foetus.*

General Form and Appearance.—Many of the features to be described here are well illustrated in Curson and Malan's (1935) chart to which reference has already been made, and in the series of photographs accompanying this chart.

At the age of 18 days the ovine embryo has the appearance, in profile, of a whitish, translucent comma, the head region being much better developed than the opposite extremity. During the next two days there is an increase in size, especially of the head region, where the primitive brain divisions are already distinguishable. At this stage the heart becomes visible as a reddish, pinhead-sized focus, situated well forward. At the twentieth day the aorta is visible, and from its caudal extremity two vessels emerge at the umbilicus to form the umbilical arteries. At about this time the dorsal region of the body is beginning to assume a more opaque appearance, thus foreshadowing the development of the vertebral column.

During the following few days the cardio-vascular system continues to develop apace, so that at the twenty-fifth day the heart has a diameter of 0·5 cm., and from it the aorta and the carotids can be seen running towards the head and towards the caudal extremity respectively. At this time the curvature of the body is so acute that the future oral regions rest between the two thoracic

limb-buds, against what is to be the sternum. The cranial region is relatively large, especially in the vicinity of the vertex, where the main divisions of the brain are plainly visible. By this time the tail has been formed, its length being approximately 2 mm.

By the end of the first month the body has become fairly plump with signs of a definitely solid axial structure in the vertebral region. The colour of the surface of the body has changed from dull translucent white to pink. The heart is still visible through the body wall, and from it vessels are seen to proceed cranially, caudally, and into the developing limbs. The ventral abdominal wall bulges outwards, presenting a herniated appearance. The length of the tail has increased to about 5 mm.

During the following week of its life the foetus undergoes rapid development and at the 38th day most of the main features of the fully developed body may be detected. The body wall becomes opaque, thus obscuring the heart region. The superficial vessels of the head, trunk, tail and limbs are visible. At this stage the cartilaginous models of the vertebrae and the scapulae show up prominently as denser white structures fairly deeply situated. The ventral abdominal bulge has disappeared.

In the middle of the second month the head is still relatively large, its size being accounted for mainly by the prominence of the crown region. At this stage the neck region begins to acquire a more definite shape. However, it is still sharply bent and the face is directed ventrally. This development continues so that at the end of the second month the neck has become fairly long, thin and well-rounded, and its ventral curvature has practically been eliminated. By this time the subcutaneous vessels, which had become very prominent and numerous, are no longer visible.

Throughout the third month the proportions of the body undergo much change, yet at the end of this period the head still appears disproportionately large, whereas the neck is relatively long and thin. However, early in the fourth month the thickness of the neck increases and by the 100th day this disproportion has to a great extent been eliminated. Now the skin over the neck has become somewhat loose and a few transverse wrinkles are to be seen. Ten days later similar wrinkles are present over the brisket and down to the carpus. The spread continues and just before birth the skin, which is now fairly thick, is comparatively loose and wrinkles are evident over the entire body.

The Head and Face.—During the first month of prenatal life the head region undergoes much development both in size and in its proportions. However, it is not until the 38th day that there are signs of facial development. At this time indications of the mouth and nostrils are detected, and by the end of another week these features are plainly visible. Towards the end of the second month the "dished" profile of the early stages (caused by the great prominence of the forehead) has practically disappeared. The facial region has increased in relative size and the mouth and nostrils are well developed. The latter are closed by epithelial plugs.

By the middle of the third month the face has come to resemble closely its definitive appearance. However, this region is still relatively small and is overshadowed by the very large and protruding forehead. At this time the rami of the mandible are well-formed, the lips are distinct and the mouth is open. The external nares are deeply excavated but are still " plugged ". The hornbuds become visible as small pinhead-sized elevations equidistant from the lateral canthus of the eye and the base of the ear.

From now on the changes are much less striking than those of the earlier period. Between the 95th and 101st days the nostrils become patent and it also becomes possible to open the mouth and to see the Anlagen of the incisor teeth. Further, the philtrum of the upper lip is distinctly visible. At the 130th day the edges of the nostrils and the upper lip, with the adjacent regions, have become smooth and bare. Ten days later the inner edge of the lower lip is serrated, while on their buccal surfaces the cheeks carry small papillae, these being best seen in the neighbourhood of the commissures of the mouth. In the foetus of 147 the Anlagen of the teeth are very prominent and the first pair of incisors appears to be on the verge of erupting.

The Eye.—At the early age of twenty-five days there is visible on the lateral surface of the head of the foetus a thin black ring 1 mm. in diameter. This is the earliest macroscopic indication of the development of the eye. As a result of the prominence of the forehead and the flexion of the neck, this ring is seen in the vicinity of the anterior limb-buds. At the end of the first month the diameter of the ring has increased slightly and the enclosed area which will later form the lens, is pale, translucent and slightly raised above the surrounding surface. At the 38th day the ring has a diameter of 3 mm., and the eyelids have commenced to grow over the developing eye. The whitish lens area is prominent. The combined effect of the straightening of the neck and the increase in relative size of the face is to place the eye in a more familiar situation. Nevertheless this organ is still somewhat closely related to the nostril and the angle of the mouth.

The black ring continues to increase both in width and in diameter. At the 45th day the latter dimension has reached 5 mm. The eyelids extend over the eye, and although they are transparent the line of fusion of the two is distinct as a denser ridge running across the eye. Just medial to the inner canthus of the eye this line is very prominent and here is foreshadowed the development of the infraorbital pouch, which, at the 52nd day, is rendered much more prominent by the curling over of the upper border of the ridge. By this time the lids have increased in thickness, and although the dark ring is still plainly visible it appears to be more deeply situated. The entire eye region is much enlarged and bulges outward from the side of the head. At the end of the second month the pouch is even more prominent and the pigmented ring has a diameter of 1·5 cm. and a thickness of 0·5 cm. Midway through the following month the ring is still larger, but by now the thickness of the lids makes accurate observation difficult. By the end of the

third month the ring has become invisible from the surface. About this time the eyelids are well defined and wrinkled, and a brownish pigmentation is seen along their borders, which are still fused.

At the 110th day it appears that the infraorbital pouch is completely developed, while the fusion of the eyelids is less definite than in previous stages. At the end of the fourth month it is possible to draw the eyelids apart, thus disclosing the apparently fully formed eye.

The External Ear.—On the 31st day the ear is visible as a small triangular flap whose base is dorsal. This flap is about 1 mm. in length. A week later the length has increased to 1·5 mm., and at the 45th day it is 2 mm. At this time there is no sign of an external acoustic meatus. Within the next seven days the length of the flap increases to 3 mm. and on it a central longitudinal ridge appears. From the basal angles of the flap low ridges extend ventrally and then converge towards each other. By the 59th day they have come together to enclose a small, roughly circular area. This enclosed area becomes depressed, forming a hollow which will develop into the external acoustic meatus. By the 66th day this depression is distinct. The flap has attained a length of 1 cm. and on either side of its central ridge an additional thickened line, parallel to the first one, is to be observed.

During the course of the next two weeks the ear increases in size and the ridges become thicker and more prominent. The length of the flap at the end of the third month is 2·5 cm., and a month later it is 4·5 cm. By this time the external meatus has become deeply excavated and to the three ridges previously mentioned two more have been added, giving the ear the appearance of a series of thickened ridges, with intervening, thinner and translucent grooves. By the end of the prenatal period the length of the flap exceeds 6 cm. and its thickness has greatly increased. At its most ventral point the rim encircling the external meatus has been deeply cut into in a V-shaped fashion. The portions lateral to this " V " have fused with the original flap in such a manner that the two resulting free borders of the pinna are practically straight.

The Genitalia.—Between the ages of 45 and 52 days it becomes possible to distinguish macroscopically between the sexes. In the male foetus of 52 days two small prominences in the inguinal region represent the scrotum. The penis is visible as a white line below the ventral abdominal surface, stretching from the ischial region to the vicinity of the umbilicus. A small prominence at the anterior extremity of this line represents the sheath. In the female of this age the developing scrotum is replaced by two small labial swellings, just anterior to which two small teats about 2 mm. long are noticed.

By the 66th day the mammary region is slightly swollen and the labial swellings have migrated caudally to a position midway between the teats and the anal region.

Midway through the second month the male foetus has developed a large whitish opaque scrotum, about 1·5 cm. in length (dorsoventral). The ventral half of the scrotum is still distinctly

divided at the median raphe into two conical lateral halves. The tips of these cones are less dense than the basal part of the sac and they have a translucent appearance. The prepuce and the penis are quite distinct, but the latter does not as yet produce any elevation of the ventral abdominal surface. One week later the length of the scrotum has increased to 2 cm.; the median groove is less distinct; the surface of the sac is smooth. At this stage one or two pairs of small teats are seen just antero-lateral to the base of the scrotum. In the female of this age the mammary region is fairly prominent, and the teats have a length of 3 mm. The labial folds have migrated further towards their definitive position, and only about one-third of the distance remains to be traversed. This is soon accomplished, and at the end of the third month the folds are finally located in the perineal region, and the formation of the vulva is well under way. The anus is well developed and prominent. The main teats are about 4 mm. long; they may be supplemented by two or four smaller, supernumerary teats.

In the male foetus of this age the scrotum is 3 cm. long and is soft and jelly-like. Teats of 2 mm. in length are visible. The prepuce is well developed and soon hairs 3 mm. in length are to be seen around the preputial opening. By the end of the fourth month the length of these hairs has reached approximately 1 cm. The surface of the scrotum, which hitherto has been smooth, becomes wrinkled. This appears to be consequent on the hollowing out and collapse of this previously solid structure.

In the female during the last month the mammae increase in size and the teats reach a length of 5 to 7 mm. The development of the vulva is completed even to the prominent ventral commissure.

The Limbs.—In foetuses of 25 days both sets of limb-buds are clearly visible. The thoracic pair are about 3 mm. in length and the pelvic ones slightly shorter. The former are divided by a central constriction into a proximal rounded, and a distal flattened part. The latter is almost fan-shaped and its surfaces face laterally and medially. In the posterior buds no constriction is evident, but towards the tip there are signs of flattening. Within the next two days these buds reach the stage of development just described for the forelimb.

By the end of the first month the length of the forelimb has increased considerably, and at the distal extremity a central groove indicates the commencing division into two digits. The lower half of the limb has rotated on its long axis in such manner that the two digits are situated medially and laterally, instead of anteriorly and posteriorly. In the posterior limb this stage has not yet been reached; the division into digits is not present, neither has the rotation commenced.

At the 38th day the digits of the forelimb are distinct and there are indications of the accessory digits. The various divisions of the limb (forearm, arm, etc.) are evident and already they have assumed their definitive relative positions. In its development the hindlimb is slightly in arrear of the forelimb.

Within a week the digits have developed considerably and the accessory digits have become plainly visible on both pairs of limbs. Close to the distal extremity of each limb a transverse, ridge-like swelling appears. It is slightly more distinct on the thoracic limb. These swellings represent the coronary bands.

During the next three weeks the fetlock region becomes moulded into shape and the coronary band becomes more prominent. However, the latter is still relatively close to the tip of the limb. By the 66th day the portion distal to the coronet has increased in size and just below the coronary band a bluish-white colouration is detected—undoubtedly this is due to the deposition of horn. The accessory digits are very prominent and are clearly defined.

The limb increases in size and the bluish colour spreads slowly downward over the surface of the third phalanx. At the 89th day the first signs of the interdigital pouches are detected. These are more advanced in the forelimb. By the end of the fourth month they appear to be fully developed.

By this time the major part of the third phalanx has been covered with horn and the line of junction of this horn with the lower dull white surface (which lies parallel to the coronary border) continues to advance distalwards. At the 130th day the horn has reached the heels, and during the next ten days the as yet uncovered triangular portion of the third phalanx becomes progressively reduced, while the horn also encroaches on the sole of the foot, the posterior half of which is covered by the 140th day. At about this time the accessory digits are almost completely covered with the same bluish-white horny substance. At the 147th day the original whitish, friable covering is confined to the tips of the hoofs and the apices of the accessory digits, the remainder of these structures being well covered with horn.

The Hair and the Wool.—Until the 38th day of foetal life there are no signs of development of hair, and the entire body surface is smooth and homogeneous. At about this time pinhead-sized white foci appear just below the surface in the region of the eyelids and the lips. By the 45th day these have increased both in size and number and are recognisable as the follicles of the tactile hairs of the face. Similar, but smaller foci appear immediately above the upper eyelids, and soon they are seen to have spread as far as the crown. These are the follicles of the ordinary hairs of the body.

During the following week the tactile follicles increase in size, while those of the ordinary hairs become more numerous and spread over the crown, but without reaching the upper part of the neck. On the lateral aspect of the shoulder and in the axilla traces of these follicles are encountered. The regions in which follicular development has occurred are not definitely demarcated; the follicles simply become smaller and more isolated towards the outskirts until they are no longer visible. At the 55th day follicles are present over the extensor surfaces of the carpus and the hock. Smaller ones are seen in the region of the flank and over the proximal portion of the thigh.

At the end of the second month the tactile follicles are large and the hairs appear to be on the point of erupting. The ordinary follicles have spread over the entire body, yet in general their distribution becomes more sparse as they are traced caudally and towards the distal ends of the limbs.

At the 66th day the tactile hairs have not yet erupted, but they have become so much increased in size that they cause small elevations on the surface. Six days later these hairs are seen emerging, at a slant, through the skin of the eyelids, the lips and the chin. By the end of the third month the longest of these hairs (on the chin) are 6 mm., while others (on upper eyelid) are only half this length.

All this time the ordinary follicles have been increasing in number and in size. By the 89th day it is possible to detect, by means of a handlens, the presence of very fine downy hairs on the forehead. Around the horn buds these hairs are arranged in whorls. They extend downwards as far as a line joining the medial canthi of the eyes. At this stage the follicles on the dorsal aspect of the carpus and along the coronet, especially close to the interdigital cleft, are large and the hairs contained in them appear to be due to erupt almost immediately. The follicles at corresponding situations on the pelvic limb are much smaller.

By the 95th day the hairs on the forehead are visible to the naked eye. They have spread over the poll and also ventralwards on to the cheeks, to the base of the ear and in the direction of the ramus of the mandible. Over the shoulders, on the lateral surfaces of the arm and forearm, over the dorsal aspect of the carpus and along the coronet, hairs just visible to the naked eye are present. With the lens it is seen that on the remainder of the forequarters, as well as over the loins, the hock and the coronet, fine hairs are just beginning to erupt. In the region of the flank and on the hindlimb (save those parts just mentioned) the follicles are densely packed and fairly large, but no eruption of hair can be detected.

At 100 days the tactile hairs have reached lengths of 0·5 to 0·8 cm. By now the forehead is covered with well-matted hair about 3 mm. long. As this is traced along the neck and back it becomes shorter, until at the loins it is only just visible. Also towards the angle of the mouth the length of the hair decreases, and on the lower part of the face it can only be seen with the help of a lens. Over the shoulders the hair has a length of between 2 and 3 mm., this also being the case on the anterior aspect of the carpus and along the coronets of the forelimb. Shorter hair (about 1 mm.) is seen on the brisket, sternum, loins, tail and proximal portions of the hocks; on the coronets of the pelvic limb it is slightly longer. On the remainder of the surface of the hindquarters hair is just visible. Fine hairs are seen on the inner surface of the ear, where they are confined to the ridges, while the depressions remain smooth and bare.

In the foetus of 110 days the tactile hairs are about 1 cm. long. On the face ordinary hairs are detectable with the naked eye. The hairs on the inner aspect of the ear have become longer and more numerous, while on the outer surface fine downy hairs are appearing.

The entire body is well covered with fairly closely packed hairs. The hairs on the coronets and those around the accessory digits have reached a length of approximately 0·5 cm.

By the end of the fourth month the body is totally covered with fairly long hairs. These are more or less straight, are fairly coarse, and have a glistening white colour. During the last month these hairs continue to increase in length, but gradually it becomes possible to distinguish below them a curly, well-matted coat, the fibres of which are finer and have a dull white colour. This appearance is detected first in the neck region from where it extends backwards over the trunk. At the end of the prenatal period this second coat, which is now recognisable as the woolly covering of the body, has practically replaced the previous hairy coat on all parts of the body excepting the lower portions of the limbs and the face.

7. *Determination of Age.*

As was pointed out in the introduction, one of the main objects of this work is to provide standards for the accurate estimation of age of Merino foetuses collected from uncontrolled sources. The necessary information is already set out in the preceding sections; but, as difficulty may arise in applying this rather detailed knowledge, it is considered advisable to extract the essential facts and to present these in a manner that will facilitate their ready application.

It is obvious that for accurate estimation one has to rely mainly upon quantitative methods. These involve the use of the regression formulae previously presented. In each of these the dimension is given in terms of age. Accordingly, the determination of the age corresponding to a measured value of a dimension necessitates a somewhat complicated calculation, for in the resulting equation the unknown (age) is present in both its first and its second powers. The solution of this equation involves factorization of rather unwieldy numbers. This process is far too elaborate to serve any useful practical purpose.

If the variables be reversed (age in terms of the dimension) the calculation is greatly simplified. From the point of view of growth this is rather illogical, for size is considered to depend on age, not age on size. However, if it will facilitate age-determination there is justification for the calculation of this "illogical" or "theoretical" formula.

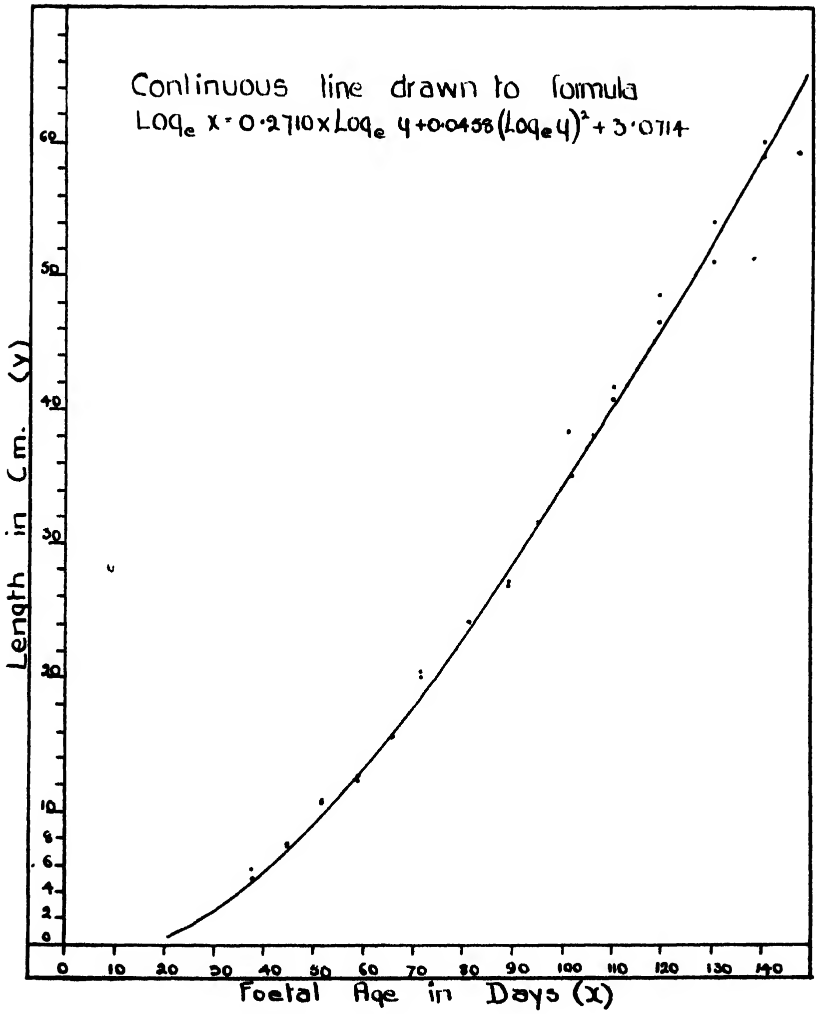
When this is done it is found that again the best-fitting curve is a parabola of the second order. Naturally the constants in the equation differ from those obtained when the same data are employed in the reverse direction. To illustrate this the formulae obtained with the vertebral column and age data are presented:—

(*a*) $\text{Log}_e\ \text{Age} = 3{\cdot}0743 + 0{\cdot}2703\ \text{Log}_e\ \text{V.C.} + 0{\cdot}0458\ (\text{Log}_e\ \text{V.C.})^2$

(*b*) $\text{Log}_e\ \text{V.C.} = -10{\cdot}4383 + 4{\cdot}3948\ \text{Log}_e\ \text{Age} - 0{\cdot}2949\ (\text{Log}_e\ \text{Age})^2$

The differences are not confined to the actual figures, but are evident also in the signs of these constants. As a result the shape of the regression line differs in the two instances. That obtained with formula (*a*) is illustrated in Fig. 41, while that of (*b*) is presented in Fig. 29.

Fig. 41.—Foetal Age in Terms of Vertebral Column Length.



From equation (*a*) age may be calculated in a direct manner. Nevertheless this computation still involves the use of logarithms and antilogarithms as well as fairly extensive multiplications which, unless a calculating machine is at hand, become very tedious. Consequently it is felt that even this "simplified" equation calls for more arithmetical labour than the average worker in biology

will be eager to undertake. Moreover, the estimation of age being in most instances merely a necessary preliminary to further investigation, it is desirable that this process should occupy the minimum of time.

It appears that graphic estimation offers a solution to the difficulties mentioned. In this method direct readings are made on any of the regression curves in Figs. 23, 25, 27, 28, 29, 33 to 37, 39 and 40; thus all calculation is eliminated. It is not necessary to confine oneself to the figures presented in this work. From the regression equation of any dimension, a series of values for this dimension at stipulated ages may be calculated. With these data a graph may be constructed to any suitable scale. Obviously increase of the latter will tend to minimise the possible error of "reading". However, it in no way affects the possible inaccuracy due to the variation inherent in the dimension. The latter error is the more important of the two, and at this stage it may be considered in some detail.

In the preceding sections the coefficients of variation of all the regression equations are stated. For convenience these figures are repeated:—

Dimensions.	*Coeff. Var.* Percentage.
Straight C.R. length	7·51
Length of Vertebral column	3·81
Length of trunk	4·07
Heart-girth	5·48
Length of tail	9·75
Length of forelimb	4·72
Length of hindlimb	4·88
Length of head	8·76
Width of head	4·75
Weight of foetus	20·07

It must be remembered that these coefficients denote the extent of variation when the dimension is calculated from age. It does not necessarily follow that when the same equation is employed for the purpose of determining age (the dimension being known) the same degree of variation will be encountered. An interesting fact is that the coefficient of variation for equation (*a*) (on page 522) is 2·6 per cent., whereas that for (*b*) is 3·8 per cent. This indicates that owing to the lesser degree of variation in the age data the determination of age from the dimension is more accurate than that of the dimension from the age. This has been proved to hold good when the entire formula is reversed; but the point at issue is whether when age is estimated from formula (*b*) the likely error will be of the order of 3·8 per cent. or of 2·6 per cent. It has been ascertained authoritatively that the exact relationship between these two coefficients of variation has not yet been solved mathematically, but it is known that this error will lie *between* 2·6 per cent. and 3·8 per cent. Visually this may be verified by noting that the horizontal deviations of the plotted points (the variation of age about the trend line) in Fig. 29, are less than the vertical deviations (the variation of vertebral column length about the same line).

It may be assumed that the same applies in the case of all the other dimensions, and that the likely error in age determination will be less than the coefficients of variation presented above. As most of these already lie well below 10 per cent. it is evident that by this graphic method age may be determined with considerable accuracy.

Naturally the dimension with the least variation will be selected for such determinations. This is the vertebral column length. However, it is suggested that the accuracy of the determination may be improved by using more than one dimension and then arriving at an "average" age. This method certainly eliminates the possibility of being misled by an extreme variation in any one dimension. Nevertheless, it would be inadvisable to determine the age of a foetus from, say, its vertebral column length and its weight, and then to take as the most likely age the exact arithmetical mean of the two values. This method would entirely lose sight of the fact that the coefficient of variation of weight is five times as large as that of the vertebral column length. It is essential that more importance be attached to the value obtained through the use of the less variable dimension. It is suggested that the relative importance of two dimensions be apportioned in the inverse ratio of their coefficients of variation. Thus, in the above example, if the age obtained by the use of weight is higher than that obtained from the vertebral column length, then the final age would be taken as the vertebral column length value plus one-fifth, or the weight value less four-fifths of the difference between these two values.

e.g. If age from weight $= x$
& age from V.C. $= y$
& $x > y$
then "true" age

$$= x - \frac{4(x - y)}{5}$$

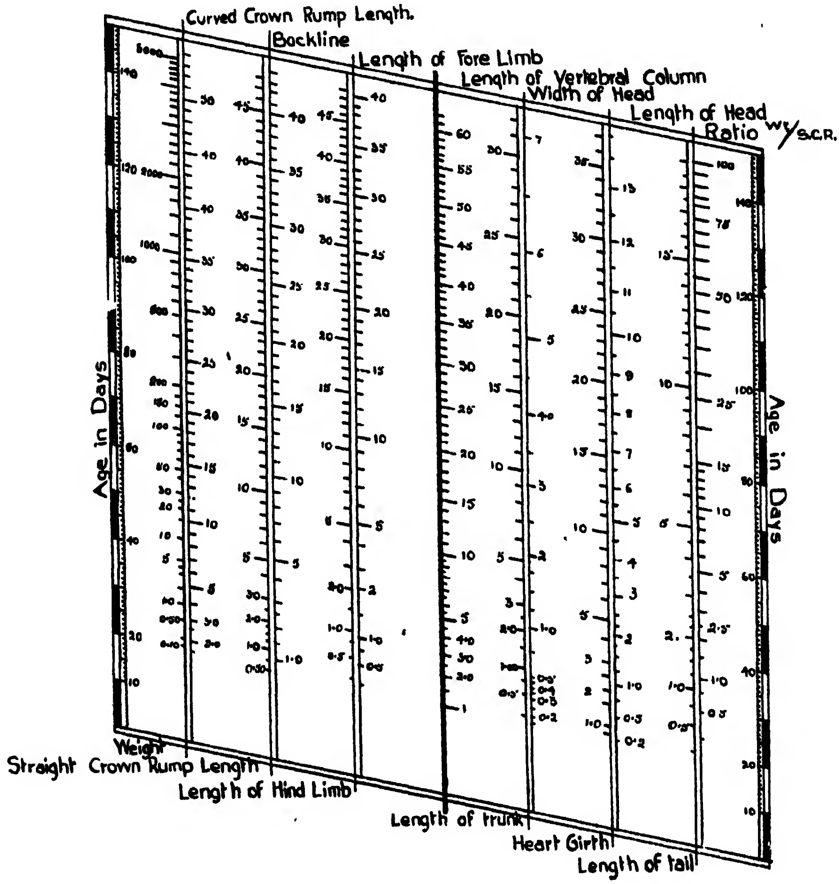
$$\text{or} = y + \frac{(x - y)}{5}$$

A further point to be mentioned is that in the choice of dimensions for ageing consideration be given to the correlation coefficient table (Table 33). Preference should be accorded to dimensions which have the least correlation with each other. Naturally their coefficients of variability must also be borne in mind, as it would be of little avail to select a highly variable dimension merely on the ground that it is not significantly correlated with any other dimension.

Although the way has now been cleared for the application in a fairly simple manner of the known quantitative data, there is still the possibility of further simplification. This is achieved by the construction, on the lines suggested by Scammon (1937), of a simple normograph (Fig. 42) in which the "expected values" of all

dimensions at any age may be read in one straight line parallel to the base of the figure. This normograph may be used for the estimation of age from one or more dimensions.

Fig. 42.—Normograph for purpose of determining either foetal age from one or more dimensions, or the values of dimensions from those of other dimensions or from foetal age.



The following procedure may be recommended:—

(1) Determine the values of the dimensions. The measurements should be made in accordance with the details supplied in Chapter 3. For each dimension two or three readings should be taken and the average of these employed. The number of dimensions to be measured should be decided by the investigator. Naturally the length of the vertebral column will be the first to receive consideration. Others suggested are trunk, heart-girth and straight crown-rump length.

(2) Mark off lightly in pencil on the respective scales the the values of the dimensions.

(3) Stretch a thread across the normograph in such a way that it lies parallel with the base, i.e. so that it intersects identical age readings on each of the vertical sides of the normograph.

(4) Keeping the thread parallel, move it either upwards or downwards until it cuts the vertebral column scale exactly at the mark indicating the value of this dimension. This scale is accorded special prominence in the centre of the figure.

(5) Observe the distribution about the thread of the points indicating the values of the other dimensions. If the majority of them display either an upward or a downward tendency, the thread should be moved slightly in the appropriate direction. Again it must be remembered that to the dimension with the lowest coefficient of variation most weight should be attached. Hence, in the example previously quoted (V.C. length and weight), the thread would be moved roughly one-fifth of the distance towards the level of the weight mark. A further mark (say trunk length), lying somewhat below the level of V.C., might tend to draw the line downward again, probably restoring it to its original position, i.e. on the V.C. mark. In the normograph the arrangement of the scales is such that the most important dimensions are closest on either side to the vertebral column scale, while farthest away are those which carry least weight.

In this way the thread is manoeuvred until the points are well distributed about it.

(6) Make sure that the line is still parallel; i.e. note the age readings on both sides. The reading on the age-scale gives the age of the foetus in days.

Some details of the above procedure may appear over-elaborate, but in actual practice it will be found that unless the majority of the other points deviate considerably (and in the same direction) from the level of the vertebral column reading, the latter will be taken as indicating the most likely age.

Having fixed the age in this quantitative manner, one would scarcely expect that any improvement would result from the use of the descriptive data listed in Table 34. However, it is recommended that in all cases one should refer to this table, if only as a safeguard against incorrect estimation of the age of an exceptionally large or small foetus. In such a case it will be found that the external appearance does not correspond with that described for a foetus of the estimated age.

In this section full directions have been given for ageing foetuses. It rests with each worker to decide for himself in how far these are to be supplied. In his decision he will be guided mainly by the nature of his investigation and the degree of accuracy considered advisable. It is suggested that for most purposes the use of the vertebral column length supplemented by a rapid reference to Table 34, will give sufficiently accurate results.

(*d*) The Maternal Mammary Gland.

For the purpose of studying the growth of this organ during the course of pregnancy the data have again been placed in monthly groups. The differences between the various groups are studied by Fisher's (1936) "analysis of variance" method. Details are presented in Table 35 and Fig. 43.

Table 35.

Weight of Mammary Gland.

Groups of Ewes.		No. of Observ.	Mean Weight.	Significance Tests.	
No.	Class.			W. Group 1.	W. Preced. Group.
			Gm.		
1	Non-pregnant..............	10	199·90	—	—
2	1st month.................	10	122·60	—	—
3	2nd month.........	8	180·13	—	—
4	3rd month.........	6	203·33	—	—
5	4th month........	7	314·71	—	—
6	5th month.................	5	806·00	XX	XX

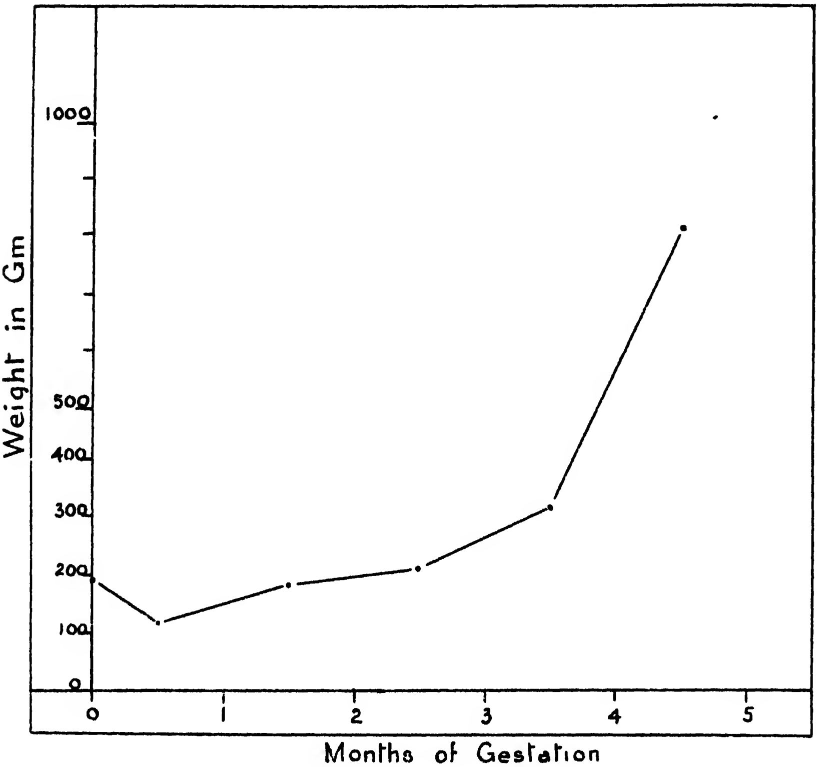
XX Significance at P = 0·01.
X Significance at P = 0·05.

The mean of the second group falls well below that of either the first or the third. This may be associated with the somewhat poorer condition at the time of slaughter of the sheep constituting this group. Nevertheless the drop is insignificant.

It is concluded that until the end of the third month there is no upward trend in the weight of the mammary gland. At about this time the weight does begin to increase, yet when the weight in the non-pregnant group is taken as the standard, this increase does not reach the level of significance until the last month of gestation. When the figure for the first month of pregnancy is employed for the purpose of comparison then the increase by the end of the fourth month is found to be just significant at P=0·05. The increase during the last month is highly significant, and by the end of gestation the initial weight has undergone a fourfold increase.

Descriptive.—During the first two months of pregnancy the appearance of the gland changes but little. In the living animal the organ can be felt as a slight swelling under the rather loose inguinal skin. The teats are small and soft. The extirpated organ has the appearance of adipose tissue and it "sets" rapidly. The organ is flattened, with a greater depth at the caudal extremity than in the cranial portion. The depth in the former region is due in part to the presence of the supramammary lymph glands which are situated here, embedded in the adipose tissue.

Fig. 43.—Weight of Maternal Mammary Gland.



Towards the end of the second month of gestation it is noticed that the diameter of the mammary bloodvessels (external pudic arteries and veins and subcutaneous abdominal veins) has been doubled. At about this time it becomes possible to withdraw from the teats small quantities of clear, watery fluid.

During the following month the size of the gland increases slightly. This increase is reflected mainly by the greater depth of the cranial part of the organ. The vessels continue to enlarge, while the teats increase in length and become very soft and flabby.

Early in the fourth month there is much more noticeable enlargement of the gland, so that the increase is evident even in the living animal. To the touch the organ is somewhat elastic, apparently on account of the accumulation of secretion within it. At this time the fluid which can be withdrawn from the teats has become " syrupy ".

By the end of the fourth month the gland is greatly enlarged and the parenchyma has a brownish colour, which contrasts with the white of the adipose tissue. The former is predominant especially in the region of the bases of the teats. The depth of the cranial part of the gland exceeds that of the caudal extremity. At this stage it is noticed that the gland does not " set " well, remaining soft and pliable for a long time after extirpation.

By the 140th day there has been even greater enlargement and the teats have become long and fairly well distended. The fluid which can be " milked " from the teat is viscid and has a creamy colour.

Just before birth this fluid becomes honey-like as regards both consistence and appearance. At this stage the bloodvessels are prominent, the veins especially being large and distended. The parenchyma of the organ is roughly cubical in shape, while adipose tissue forms but a narrow peripheral rim.

Thus it is found that the mammary gland undergoes most of its macroscopic development during the second half of pregnancy. This is in agreement with the findings of Hammond (1927) in the case of the cow, i.e. that during the first half of pregnancy there is first an organization of the collecting system, and then growth of the secreting tissue, while during the second half secretion commences and, as a result of the accumulation of the secretory product, the size of the organ is increased.

(*c*) Maternal Endocrine Glands.

It is of interest to investigate the possibility of detecting in these glands macroscopic changes which may be associated with the physiologic state of pregnancy. Attention has been directed mainly to the changes in weight. This is due to the fact that in most of these organs linear measurements are impracticable, while volume determinations (by the displacement method) on these small glands call for more time than could be allowed to lapse before sectioning and the initiation of fixation for later histological examination.

In view of the desirability of having in Group 1 only ewes in the same phase of sexual life, all data from sheep judged to have been in anoestrum have been excluded. Thus this group comprises only ewes at various stages of the dioestrus cycle.

Hypophysis.—Details of the mean weights of the various groups are presented in Table 36.

TABLE 36.

Weight of Hypophysis.

Groups of Ewes. No.	Groups of Ewes. Class.	No. of Observ.	Mean Weight of Hypophysis.
			Gm.
1	Non-pregnant.............	8	0·8125
2	1st month................	11	0·6682
3	2nd month................	8	0·7438
4	3rd month................	5	0·7700
5	4th month................	7	0·7286
6	5th month................	5	0·9600

It is found that none of the differences between groups is significant even at the 5 per cent. level of probability. When the data are placed in two groups only (pregnant and non-pregnant) the differences are still insignificant.

In view of these findings no attempt has been made to analyse the data relating to length, width and depth of this organ.

Thyroid, Adrenals and Epiphysis.—For these organs fewer data are available, hence the method of grouping has been altered, group 2 comprising the former groups 2, 3 and 4, while the former nos. 5 and 6 have been amalgamated to form the third group of this section.

Details of these glands are tabulated below (Table 37). Not in a single instance is the difference between groups significant at P 0·05. Even reduction of the number of groups to two (pregnant and non-pregnant) fails to disclose any significant difference.

TABLE 37.

Weights of Thyroid, Adrenal and Pineal.

Groups of Ewes. No.	Groups of Ewes. Class.	No. of Observ.	Mean Weight. Thyroid.	Mean Weight. Adrenal.	Mean Weight. Pineal.
			Gm.	Gm.	Gm.
1	Non-pregnant................	5	2·82	3·24	0·11
2	1st, 2nd and 3rd month........	8	1·98	2·93	0·14
3	4th and 5th month............	12	2·42	3·52	0·14

Corpus Luteum.—For the study of this temporary endocrine gland recourse is had to a rather indirect method. The weights of the ovaries have been recorded, and by studying these it is hoped to gain some information regarding the changes in weight of the corpus luteum. Again the sheep are placed in six groups and in each of these two sub-groups are formed, comprising respectively

the data of the ovary with the corpus luteum (C.L.) and those of the ovary without this body (N.C.L.). In Table 38 details will be found.

TABLE 38.

Weights of Ovaries.

Groups of Ewes.		No. of Observ.	Mean Ovarian Weight.	
No.	Class.		Ovary with C.L.	Ovary without C.L.
			Gm.	Gm.
1	Non-pregnant..............	9	1·40	0·69
2	1st month.................	12	1·38	0·66
3	2nd month.................	8	1·44	0·73
4	3rd month.................	6	1·33	0·67
5	4th month.................	7	1·14	0·61
6	5th month.................	5	1·00	0·58

On analysis it is found that none of the differences between groups of the C.L. series is significant. In view of the fact that the corpus luteum accounts for practically half the weight of the ovary in which it is located (see difference between the two ovaries of the same sheep), it is maintained that any change in size of the corpus will be reflected in the weight of the ovary. Consequently the above finding suggests that throughout pregnancy there is no significant change in the weight of the corpus luteum. However, there is the possibility that changes of a compensating nature may occur in both the corpus and the remainder of the ovary. However, reference to the N.C.L. series shows that here no significant changes have occurred. Moreover, the downward trend in the last two groups is well reflected in both series. This may be ascribed to progressive atresia of Graafian follicles, and the somewhat greater drop in the C.L. series may be attributed to this effect being more marked in the ovary carrying the corpus luteum (Hammond, 1927).

From the above reasoning it is concluded that, at least in the present data, there is no evidence of any change in the weight of the corpus luteum throughout the course of pregnancy.

Regarding all the endocrines it must be stated that no significant differences have been demonstrated. Probably the reason for this is that, considering the great variations encountered, the numbers of observations were too limited to allow of the detection of any changes that might have taken place. Probably the main value of this section lies in the fact that it demonstrates the futility of such studies on any but very extensive groups of animals.

(*f*) GENERAL.

1. In the review of the literature it has been pointed out that the ovum may either become implanted in the horn on the same side as the ovary from which it originated, or it may migrate to

the opposite side. Attention is directed to the statement of Clark (1936) that in the bovine more foetuses are carried in the right horn than in the left, and that this is not due to more frequent functioning of the right ovary, but to more frequent migration from left to right. Considering the topography of the rumen one might conclude that the pressure exerted by this organ has an important bearing on these findings. The same would be expected to apply to the sheep.

The data collected during the course of the present work are presented in Table 39.

TABLE 39.

Situation of Foetus and Corpus Luteum.

	No Migration.	Migration.	Total.
Foetus in Right Horn................	18	3	21
Foetus in Left Horn................	10	5	15
TOTAL................	28	8	36

By means of the X^2 test it is possible to compare with each other migration and non-migration; migration to the right and migration to the left; foetus in right horn and foetus in left. Only in the first instance (migration and non-migration) do the observed figures differ significantly from their expected values. Thus it is seen that there is a very definite tendency for the ovum to become implanted on the side on which it is liberated. In fact, the odds are more than 100 to 1 against its migration. There is no significant difference regarding the number of foetuses carried in each horn, nor is migration more likely to occur in one direction than in the other.

In view of the relatively small number of animals employed it has been decided to include here the data supplied by Curson (1934). The details for the combined group of 72 ewes are set out in Table 40.

TABLE 40.

Situation of Foetus and Corpus Luteum.

	No Migration.	Migration.	Total.
Foetus in Right Horn................	37	5	42
Foetus in Left Horn................	25	5	30
TOTAL................	62	10	72

Although the difference between "total right" and "total left" appears to have been increased, it does not yet reach significance. It will be seen that migrations have occurred with equal frequency in each direction. Further, the preponderance of non-migrations over migrations is even greater here than in the previous table. Thus it is seen that the results obtained with the larger group of animals are practically identical with those of the present series alone. Therefore, one may conclude that in the sheep there is no special tendency towards migration in one direction, nor is there a tendency for the foetus to lie more frequently in one horn than in the other. Almost invariably will the foetus be situated in the horn corresponding to the ovary with the corpus luteum. Migration takes place but rarely, the odds against this occurrence being fairly long.

2. At times it might be useful to be able to differentiate between the true weight of a pregnant ewe and the weight of the foetal system. In order to arrive at the correction to be applied to the gross weight, it is necessary to study the growth of the foetal system. As it was felt that the weight of the ewe might have some definite bearing on the weight of the foetal system, it was decided to incorporate this variable. However, Curson and Malan's (1937) method of reducing the weight of the foetal system to a percentage of the nett weight of the ewe (gross weight less weight of foetal system) does not appear to be justified, as it presumes a proportionality which has not yet been proved to exist. The more satisfactory method is to determine the equation for weight of the foetal system in terms of gestation age and weight of ewe. This equation is

$$\text{Log}_e \text{ Foetal system} = -4 \cdot 5176 + 2 \cdot 3048 \text{ Log}_e \text{ Gestation Age} + 0 \cdot 5105 \text{ Log}_e \text{ Nett Wt. of ewe.}$$

Weight F.S. in gm., Gestation Age in days and Weight of ewe in Kg.

The coefficient for gestation age is highly significant, but that for weight of ewe is definitely insignificant. This means that it has not been possible to demonstrate that the weight of the ewe has any significant effect upon the weight of the foetal system. It must be pointed out that the ewes were all of fairly even size and that the weight of the foetal system is subject to large variation. These two factors may account for the insignificant result.

However, having failed to demonstrate significance as regards nett weight of the ewe, there is no justification for the retention of this variable. Thus the equation is simplified by determining the weight of the foetal system in terms of gestation age:—

$$\text{Log}_e \text{ F.S.} = -3 \cdot 0337 + 2 \cdot 3891 \text{ Log}_e \text{ Gestation Age.}$$

The coefficient of variation is high, namely 26·87 per cent.

Having obtained the weight of the foetal system, the nett weight of the ewe is obtained with ease:—

$$\text{Nett weight} = \text{Gross Wt.} - \text{Wt. F.S.}$$
$$= \text{Gross Wt.} - \text{Antilog } (-3 \cdot 0337 + 2 \cdot 3891 \text{ Log}_e \text{ Gest. Age}).$$

As only data relating to sheep pregnant for 31 days or longer have been employed, the above equation holds good only from the beginning of the second month of gestation. During the first month the weight of the foetal system is negligible.

CHAPTER 5.—SUMMARY.

Attention is directed to the paucity of information regarding prenatal growth of domesticated mammals and to the fact that much of the available knowledge is rendered practically valueless through lack of accurate definition. The importance of accurate standards for ageing foetuses is stressed. The present investigation aims at providing such standards for the Merino sheep. In addition, during the course of the work valuable data have been collected concerning those maternal systems which are chiefly associated in the reproductive process, i.e. the genitalia, the endocrines and the mammary glands. Wherever possible, changes of a qualitative nature have been described in detail, while quantitative data have been subjected to statistical analysis.

In the sheep it is found that, with the exception of the Fallopian tubes, the entire genital tract undergoes extensive enlargement during pregnancy. In the horns and the body of the uterus a significant enlargement is evident sooner than in the vagina and the cervix. Whereas the former have to carry the developing foetal system, increase in the latter merely anticipates the needs of parturition. In all these organs the enlargement observed is the result of active growth of the regions concerned, not of mere passive stretching.

By about mid-term the placenta has reached its maximum development. After this there is a fall in weight, while the individual cotyledons tend to become flattened. Throughout pregnancy the weight of the foetal membranes increases steadily.

The total volume of foetal fluid increases rapidly until the third month, remains practically unchanged during the fourth, and then again increases. The first increase is the result of rapid accumulation of amniotic fluid, while the second is due entirely to the allantoic fluid; for during the second half of gestation the volume of amniotic fluid becomes decreased. During the fourth month a balance is attained between allantoic increase and amniotic decrease. This flattening of the curve is reflected in most of the weights and measurements of the uterus. Although specific gravity and hydrogen-ion concentration of the fluids were considered, no important changes were detected.

The weight of the maternal mammary gland begins to increase in the fourth month of gestation, but the major portion of the increase occurs only in the last month. The watery secretion observed at mid-term changes gradually into the yellowish honey-like cholostral milk.

Regarding the maternal endocrine glands it is concluded that as a result of large individual variations no significant macroscopic changes can be demonstrated. For such studies very large groups of animals would be necessary.

With reference to the foetus it is found that growth both in weight and in length follows a double parabolic trend represented by the formula

$$\mathrm{Log}_e \text{ Dimension} = a + b \ \mathrm{Log}_e \text{ Age} + c\ (\mathrm{Log}_e \text{ Age})^2.$$

The coefficient of variation for weight is over 20 per cent., whereas that of the most satisfactory length measurement (length of the vertebral column) is under 4 per cent.

The percentage growth-rates of both length and weight decrease steadily and continuously throughout prenatal life. There is no evidence of abrupt " breaks " in these rates.

The weight-length ratio of the growing foetus follows a simple logarithmic trend. The coefficient of variation is high, namely about 17 per cent.; hence this ratio has little value as an index of age.

In connection with regional growth it is found that with the exception of length of the neck (which is highly variable) all the dimensions considered are capable of representation by parabolae such as described for growth of the entire body. From a consideration of the percentage growth-rates of these dimensions the theory is formed that changes in the proportions of the growing foetus are the result of differential rates of retardation of regional growth. The degree of retardation in any region appears to be proportional to the lapse of time since the inception of growth in that region.

In one comprehensive table are set out the total, trend and residual correlation coefficients for each pair of dimensions. In all instances the total correlation is highly significant, but only between closely related dimensions is there a significant residual correlation. Thus in most instances the high total correlation is due almost entirely to the common growth-trend.

As the object of the work is to provide ageing standards, details are furnished as to the manner in which the above knowledge is to be applied. Special mention is made of a simple normograph so constructed as to allow of the straightforward reading of age from one or more measured dimensions.

In a supplementary section are considered the relation between the pregnant horn of the uterus and the situation of the corpus luteum, and the growth of the entire foetal system. In connection with the former it is concluded that there is a significant tendency for the foetus to be carried in the horn on the same side as the ovary containing the corpus luteum. The numbers of foetuses carried in each horn do not vary significantly, neither is there evidence of any tendency towards migration of the ovum in one special direction.

Knowledge of the weight of the foetal system is of importance to workers wishing to adjust the live weights of pregnant ewes. In this investigation the nett weight of the ewe (i.e. gross weight less weight of foetal system) is found to have no significant effect on the weight of the foetal system. Hence an equation is presented in which the weight of the foetal system (i.e. the correction to

be applied) is given in terms only of the length of gestation. However, as the coefficient of variation is over 26 per cent., the correction must be regarded as very approximate.

BIBLIOGRAPHY.

ADDISON, W. H. F., AND M. ADAMS (1926). A comparison, according to sex, of the relative weights of three parts of the hypophysis in the albino rat. *Anat. Rec.*, Vol. 33, No. 1, pp. 1-12.

ANDERSEN, D. H. (1933). Weight of the pituitary and the thyroid of the rat at various stages of the oestrus cycle. *Proc. Soc. Exp. Biol. Med.*, Vol. 30, No. 5, pp. 657-659.

ANDERSEN, D. H., AND H. S. KENNEDY (1932). Studies on the physiology of reproduction IV. Changes in the adrenal gland of the female rat associated with the oestrus cycle. *J. Physiol.*, Vol. 76. No. 2, pp. 247-260.

ANDERSEN, D. H., AND H. S. KENNERY (1933A). Studies on the physiology of reproduction V. The adrenal cortex in pregnancy and lactation. *Ibid.*, Vol. 77. No 2, pp 159-173.

ANDERSEN, D. H., AND H. S. KENNEDY (1933B). The effect of gonadectomy on the adrenal, thyroid and pituitary glands. *Ibid.*, Vol 79, No. 1, pp. 1-30.

AREY, L. B. (1934). *Developmental Anatomy*, 3rd edit.. W. B. Saunders & Co., Philadelphia and London.

ASDELL, S. A. (1925). The inception of lactation in the cow and the goat. *J. Agric. Sci.*, Vol. 15. No. 3, pp. 358-374.

ASSHETON, R. (1903). *Cit.* Assheton (1905).

ASSHETON, R. (1905). The morphology of the ungulate placenta, particularly the development of the organ in the sheep, and notes upon the placenta of the elephant and hyrax. *Phil. Trans. Roy. Soc.*, London. Series B. Vol. 198, pp. 143-220.

BAILEY, F. R., AND A. M. MILLER (1921). *Text-book of Embryology*. 4th edit. William Wood & Co., New York.

BARCROFT, J., AND P. ROTHSCHILD (1932). The volume of blood in the uterus during pregnancy. *J. Physiol.*, Vol. 76, No. 4, pp. 447-459

BARCROFT, J., W. HERKEL, AND S. HILL (1933). The rate of blood-flow and gaseous metabolism of the uterus during pregnancy. *Ibid.*, Vol 77. No. 2. pp. 194-206.

BARKER, D. B. (1937). Studies of the suprarenal glands of dogs. I Comparison of the weights of suprarenal glands of mature and immature male and female dogs. *Am. J. Anat.*, Vol. 60. No. 2, pp. 231-252.

BARRY, L. W. (1920). The effects of inanition on the pregnant albino rat, with especial reference to the changes in the relative weights of the various parts, systems and organs of the offspring. *Anat. Rec.*, Vol. 18, No. 3, p. 221.

BARTLETT, S. (1926*a*). Variations in the live weight of dairy cows. *J. Agric. Sci.*, Vol. 16, No. 3, pp. 383-391.

BARTLETT, S. (1926*b*). The effect of pregnancy on the live weight of dairy cows. *J. Agric. Sci.*, Vol. 16, No. 3, pp. 392-405.

BEER, G. (1925). Beitrag zur Frage der Entwicklung der somatischen Geschlechtsmerkale beim Rinderfotus. (Abstr.) *Jahresbericht Vet. Med.*, Vol. 45, p. 237.

BEHNE, W. (1929). Pathologisch-histologische Untersuchungen über die Wandveranderungen der Uterusgefase trachtig gewesener Schafe. *Inaug. Diss.*, Leipzig.

BERGMANN, R. (1922). Beitrage zur Alterbestimmung vom Kalbsfoten der schwarzbunten Niederungsrasse. *Arch. Wissensch. Praktisch. Tierheilk.*, Vol. 47, pp. 292-318.

BONNET, R. (1895). *Cit.* Assheton (1905).

BONNET, R. (1898). *Cit.* Assheton (1905).

BRODY, S. (1927*a*). Growth and Development, III. Growth rates, their evaluation and significance. *Univ. Mo. Agric. Exp. Sta. Res. Bull.*, No. 97.

BRODY, S. (1927*b*). Growth and development, IV. Growth rates during the self-accelerating phase of growth. *Ibid.*, No. 98.

BRODY, S. *et al.* (1926). Growth and development. 1. Quantitative data. (by the growth committee). *Ibid.*, No. 96.

BRODY, S., AND A. C. RAGSDALE (1926). Time changes in milk secretion. *Ibid.*, No. 96, pp. 41-45.

BRODERMANN, H. (1921). Untersuchungen über Gewicht des Gravinden Uterus, der Eihullen, der Foten und über Fruchtwassermengen bei Schweinen. *Inaug. Diss.*, Hanover.

CARMICHAEL, W. J., AND J. B. RICE (1921). Variation in farrow with special reference to the weight of pigs. *Illinois Agric. Exp. Sta. Bull.*, No. 226.

CLARK, C. F. (1936). Does the right ovary of the bovine function more frequently than the left *J. Am. Vet. Med. Assoc.*, Vol. 88, No. 1, pp. 62-65.

CLEVELAND, R., AND J. M. WOLFE (1933). Cyclic histological variations in the anterior hypophysis of the sow (*sus scrofa*). *Am. J. Anat.*, Vol. 53. No. 2. pp. 191-220.

COLIN, G. (1888). *Cit.* Needham.

CRAIG, J. F. (1912). *Flemming's Veterinary Obstetrics.* 3rd. Edit. Bailliere, Tindall & Cox, London.

CRUICKSHANK, E. M. (1929). Observations on the iodine content of the thyroid and ovary of the fowl during the growth, laying and moulting periods. *Biochem. J.*, Vol. 23, pp. 1044-1049.

CURSON, H. H. (1934). Studies on sex physiology XI. The relationship between a corpus luteum verum and the corresponding pregnant horn. *Onderstepoort J. Vet. Sci. & Anim. Industry*, Vol. 3, No. 1, pp. 133-134.

CURSON, H. H. (1937). Personal communication.

CURSON, H. H., AND A. P. MALAN (1936). The extent of the gravid Merino uterus in relation to the vertbral column in the dorsal recumbent position, and the weights of the gravid uterus and foetus in relation to the general body weight. *Onderstepoort J. Vet. Sci. & Anim. Industry*, Vol. 7, no. 1, pp. 261-274.

CURSON, H. H., AND A. P. MALAN (1935). Studies in sex physiology No. 13. The changing proportions of the Merino lamb from the second to the fifth month of prenatal life. *Ibid.*, Vol. 4, No. 2, pp. 481-492.

CURSON, H. H., AND G. S. MARE (1934). Studies in sex physiology No. 12. The situation of the developing foetus and the relationship between the pregnant horn and the corresponding corpus luteum verum. *Ibid.*, Vol. 3, No. 2, pp. 477-486.

CURSON, H. H., AND J. B. QUINLAN (1934). Studies in sex physiology No. 10. The situation of the developing foetus in the Merino sheep. *Ibid.*, Vol. 2, No. 2, pp. 657-663.

DEANESLEY, R. (1928). A study of the adrenal cortex in the mouse and its relation to the gonads. *Proc. Roy. Soc.*, London, Series B, Vol. 103, pp. 523-546.

DONALD, H. P., AND J. W. McCLEAN (1935). The growth-rate of lambs in Canterbury—a preliminary study of the variation in growth-rate of lambs in Canterbury. *New Zealand J. Sci. and Techn.*, Vol. 17, pp. 497-519.

DONALDSON, H. H. (1924). *The Rat*. 2nd edit. Memoirs of the Wistar Institute of Anatomy and Biology. Philadelphia.

DRAPER, R. L. (1920). The prenatal growth of the guinea pig. *Anat. Rec.*, Vol. 18, No. 4, pp. 369-392.

DUERDEN, J. E., AND M. I. F. RITCHIE (1924). Development of the Merino fibre. *S. African J. Sci.*, Vol. 21, pp. 480-497.

DU TOIT, P. J., AND J. H. R. BISSCHOP (1929). The breeding of cattle on phosphorus deficient veld *15th Rep. D.V.S., Un. of S.A.*, pp. 1059-1166.

ELLENBERGER, W., AND H. BAUM (1921). *Handbuch der Vergleichenden Anatomie der Haustiere.* August Hirschwald, Berlin

ECKLES, C. H. (1919). A study of the birth weight of calves. *Univ. Mo. Agric. Exp. Sta. Res. Bull.*, No. 35.

ECKLES, C. H. (1920). Normal growth of dairy cattle. *Ibid.*, No. 36.

FAVILLI, N. (1928). L'utero dei ruminanti struttura normale e suoi adattamenti alla gravidanza. (Abstr.) *Jahresbericht Vet. Med.*, Vol. 49, No 1, p. 59

FAVILLI, N. (1929). Variazioni fisiche dell 'utero gestante. Contributio critico e sperimentale. (Abstr.) *Ibid.*, Vol. 49, No. 1, p. 60.

FISHER, R. A. (1936). *Statistical Methods for Research Workers.* 6th Edit. Oliver & Boyd, Edinburgh and London.

FITCH, J. B., P. C. McGILLIARD, AND G M. DRUMM (1924). A study of the birth weight and gestation of dairy animals. *J. Dairy Sci.*, Vol. 7, No. 3, pp. 222-233.

FREUDENBERGER, C. B. (1932). A comparison of the Wistar albino and the Long-Evans hydrid strain of the Norway rat. *Am. J. Anat.*, Vol. 50, No. 2, pp. 293-349.

GALPIN, N. (1935). The prenatal development of the coat of the New Zealand Romney lamb. *J. Agric. Sci.*, Vol. 25, No. 3, pp. 344-360.

GROENEWALD, J. W. (1935). The influence of rations low in certain minerals on the composition of the blood and milk of cows, and on the blood of their progeny. *Onderstepoort J. Vet. Sci. and Anim. Industry*, Vol. 4, No. 1, pp. 93-165.

GUGGISBERG, VON H. (1933). Schildruse und Schwangerschaft. *Endokrinologie*, Vol. 13, No. 2, pp. 73-79.

GURLT, O. (1847). *Cit.* Needham.

HACKERSCHMIDT, H. (1920). Histologische beitrage zur Kenntnis der involution des normalen Uterus der Zeige. *Inaug. Diss.*, Leipzig.

HAMMOND, J. (1927). *The Physiology of Reproduction in the Cow.* Cambridge University Press.

HAMMOND, J. (1932). *Growth and Development of Mutton Qualities in the Sheep.* Oliver & Boyd, Edinburgh and London.

HAMMOND, J. (1937). Pregnancy and nutrition of the embryo in the rabbit. *The School Sci. Rev.*, No. 32, pp. 548-557.

HATAI, S. (1913). On the weights of the abdominal and the thoracic viscera, the sex glands, ductless glands and the eyeballs of the albino rat (mus norvegicus albinus) according to body weight. *Am. J. Anat.*, Vol. 15, No. 1, pp. 87-119.

HENNING, W. L. (1937). A double sheep pregnancy with a single corpus luteum. *J. Heredity*, Vol. 28, pp. 61-62.

HERRING, P. T. (1920). The effect of pregnancy upon the size and weight of some of the organs of the body. *Br. Med. J.*, Vol. 11, p. 886.

HILTY, H. (1908). Untersuchungen uber die Evolution und die Involution der Uterusmucosa vom Rind. *Schweiz. Arch. f. Tierheilk.*, Vol. 50, No. 5, pp. 268-323, and No. 6, pp. 353-406.

HOFFMAN, F. E. (1935). Ewes and lambs: their care and management. *Farming in South Africa*, June 1935.

HOLZGRUBER, T. (1925). Beobachtungen von Pferdegeburten. (Abstr.) *Jahresbericht Vet. Med.*, Vol. 45, p. 265.

HUMPHREY and KLEINHEINZ, (1908). *Cit.* Hammond (1932).

JACKSON, C. M. (1909). On the prenatal growth of the human body and the relative growth of the various organs and parts. *Am. J. Anat.*, Vol. 9, No. 1, pp. 119-165.

JACKSON, C. M. (1913). Postnatal growth and variability of the body and of the various organs in the albino rat. *Am. J. Anat.*, Vol. 15, No. 1, pp. 1-68.

JONES, S. V. H., AND J. E. ROUSE (1920). The relation of age of dam to observed fecundity in domesticated animals. 1: Multiple births in cattle and sheep. *J. Dairy Sci.*, Vol. 3, pp. 260-290.

JOUBERT, P. J. (1936). The milk production of sheep and its effect on the growth of lambs. *Thesis for M.Sc. (Agric.)*. Univ. of Pretoria.

KALTNER, A. (1923). Studien uber das Corpus luteum beim Rind. *Zbl. f. Gynakol.*, Vol 47, pp. 1449-1451.

KAPPERS, A. J. (1936). Brain—bodyweight relation in human ontogenesis. *Proc. Roy. Acad., Amsterdam*, Vol. 39, pp. 871-880.

KEEFER, W. O. (1937). Intra-uterine location of bull and heifer calves. *Vet. Med.*, Vol. 32, No. 7, p. 327.

KELLER, K. (1920). Über somatische Geschlechtsmerkmale beim Rinderfetus. *Wien. Tierarz. Monatchr.*, Vol. 7, pp. 137-145.

KING, H. D. (1915). On the weight of the albino rat at birth and the factors that influence it. *Anat. Rec.*, Vol. 9, pp. 213-231.

KISLOVSKY, D. A., AND B. A. LARCHIN (1931). The periods of embryonic growth in cattle. *J. Agric. Sci.*, Vol. 21, No. 4, pp. 659-668.

KRAFT, H. (1923). Histologische untersuchungen uber die Involution des normalen Uterus des Rindes mit besonderer Berucksichtiging des elastischen Gewebes. (Abstr.) *Jahresbericht Vet. Med.*, Vol. 43, p. 223.

KUPFER, M. (1923). Beitrage zur Morphologie der weiblichen Geschlechtsorgane bei Saugetieren. *Vierteljahrschrift der naturforschenden Gesellschaft, Zurich,* No. 68.

KUPFER, M. (1936). The early stages of body-form development in cattle. *Farming in South Africa,* May 1936.

LAMBERT, I. (1928). Uber den bau des Eileiters der Haussaugtiere. (Abstr.) *Jahresbericht Vet. Med.*, Vol. 49, No. 1, p. 58.

LANE-CLAYPON, J. E., AND E. H. STARLING (1906). An experimental enquiry into the factors which determine the growth and activity of the mammary glands. *Proc. Roy. Soc.*, London, Series B. Vol. 77, pp. 505-522.

LEE, F. C. (1929). The tubo-uterine junction in various animals. *Vet. J.*, Vol. 85, pp. 16-34.

LEOPOLD (1888). *Cit.*, Hammond (1927).

LOEB, L., AND C. HESSELBERG (1917A). The cyclic changes in the mammary gland under normal and pathological conditions. 1: the changes in the non-pregnant guinea-pig. *J. Exp. Med.*, Vol. 25, No. 2, pp. 284-304.

LOEB, L., AND C. HESSELBERG (1917B). The cyclic changes in the mammary gland under normal and pathological conditions. 2: The changes in the pregnant guinea-pig, the effect of lutein injections, and the correlation between the cycle of the uterus and ovaries and the cycle of the mammary gland. *Ibid.*, Vol. 25, No. 2, pp. 305-322.

LOCHHEAD, J., AND W. CRAMER (1908). The glycogenic changes in the placenta and the foetus of the pregnant rabbit: A contribution to the chemistry of growth. *Proc. Roy. Soc.*, London, Series B, Vol. 80, pp. 263-284.

LONG, J. (1906). *Cit.*, Lowry.

LOWREY, L. G. (1911). Prenatal growth of the pig. *Am. J. Anat.*, Vol 12, No. 2, pp. 107-138.

MACDOWELL, E. C., E. ALLEN, AND C. G. MACDOWELL (1927). The prenatal growth of the mouse. *J. Gen. Physiol.*, Vol. 11, No. 1, pp. 57-70.

MALAN, A. P., AND H. H. CURSON (1936A). Studies in sex physiology, No. 15. Further observations on the body weight and crown-rump length of Merino foetuses. *Onderstepoort J. Vet. Sci. and Anim. Industry*, Vol. 7, No. 1, pp. 239-249.

MALAN, A. P., AND H. H. CURSON (1936B). Studies in sex physioilogy, No 16. Surface area in the foetus of the Merino sheep. *Ibid.*, Vol. 7, No. 1, pp. 251-259.

MALAN, A. P., AND H. H. CURSON (1937). Studies in sex physiology, No 18. On the growth of the gravid uterus in the Merino. *Ibid.*, Vol. 8, No. 2, pp. 417-427.

MALL, F. P. (1910). Section in *Manual of Human Embryology*, Edited by F. Keibel and F. P. Mall. J. B. Lippincot Co., Philadelphia and London.

MARAIS, I. P. (1936). Untersuchungen uber den Sexualzyklus bei Merinoschafen. *Inaug. Diss.*, Leipzig.

MARZA, V. D., AND A. V. BLINOV (1936). L'hypophyse anterieure et la thyroide au cours de l'ovulation. *Comp. Rend. Soc. Biol.*, Vol. 121, p. 1438.

MINOT, C. S. (1908). *Cit.*, Brody (1927a).

MUMFORD, F. B. (1901). *Cit.*, Hammond (1932).

MURRAY, J. A. (1921). Normal growth in animals. *J. Agric. Sci.*, Vol. 11, No. 3, pp. 258-274.

MUTOW, T. (1937). Influence of gestation, lactation and some pathological conditions on the weight of the suprarenal glands in the albino rat. (Abstr.) *Physiol. Abstr.*, Vol. 22, Nos. 6 and 7, p. 684.

NAHM, L. J., AND F. F. McKENZIE (1937). The pituitary glands of ewes in various phases of reproduction. *Univ. Mo. Agric. Exp. Sta. Res. Bull.*, No. 257.

NEEDHAM, J. N. (1931). *Chemical Embryology*, 3 Vols. Cambridge University Press.

NOBACK, G. J. (1922). Simple methods of correlating crown-rump and crown-heel lengths of the human fetus. *Anat. Rec.*, Vol. 23, No. 4 pp. 241-244.

NOBACK, G. J. (1925). The lineal growth of the respiratory system during fetal and neonatal life is expressed by graphic analysis and empirical formulae. *Am. J. Anat.*, Vol. 36, No. 2, pp. 235-274.

OPPERMANN (1922). *Cit.*, Hammond (1927).

ORYWALL, W. (1933). Anatomische untersuchungen an der norddeutschen und suddeutschen Rattenschilddruse unter besonderer Berucksichtigung der Jodwirkung. *Virch. Arch. Path. Anat.*, Vol. 287, p. 348.

PATON, D. N., B. P. WATSON, AND J. KERR (1907). On the source of the amniotic and allantoic fluids. *Trans. Roy. Soc.*, Edinburgh, Vol. 46, No. 3, pp. 71-102.

QUINLAN, J., AND G. S. MARE (1931). The physiological changes in the ovary of the Merino sheep in South Africa, and their practical application in breeding. *17th Rep. D. V. S. and Anim. Industry, Un. of S.A.*, pp. 663-703.

QUINLAN, J., G. S. MARE, AND L. L. ROUX (1932). The vitality of the spermatozoon in the genital tract of the Merino ewe, with special reference to its practical application in breeding. *18th Ibid.*, pp. 831-870.

QUINLAN, J., AND L. L. ROUX (1936). Researches into sterility of cows in South Africa. The influence of: (1) dry rations, (2) lack of exercise, and (3) lack of sunlight on reproduction in beef heifers and cows. *Onderstepoort J. Vet. Sci. and Anim. Industry*, Vol. 6, No. 2, pp. 719-772.

RAGSDALE, A. C., C. W. TURNER AND S. BRODY (1924). The effect of gestation upon lactation in the dairy cow. *J. Dairy Sci.*, Vol. 7, pp. 24-30.

RAGSDALE, A. C., E. C. ELTING AND S. BRODY (1926). Weight growth and linear growth. *Univ. Mo. Agric. Exp. Sta. Res. Bull.*, No. 96, pp. 7-40.

RASMUSSEN, A. T., AND R. HERRICK (1922). A method for the volumetric study of the human hypophysis cerebri with illustrative results. *Proc. Soc. Exp. Biol. Med.*, Vol. 19, pp. 416-423.

RICHTER, R. (1936). Beitrag zur kenntnis des juvenilen und des gravid gewesenen Uterus des Schweines. *Inaug. Diss.*, Leipzig.

RIDDLE, O., AND T. C. NUSSMANN (1933). A sex difference in pituitary size and intestinal length in doves and pigeons. *Anat. Rec.*, Vol. 57, pp. 197-204.

RORIK, H. H. (1907). Berechnung der Oberflache der Uteruskarunkeln (semiplacenta materna) beim Rind. *Arch. Wissensch. Prakt. Tierheilk.*, Vol. 33, Nos. 4 and 5, pp. 421-431.

ROSENBLUETH, A., AND R GAYET (1932). The failure of diet to influence the adrenin content of the adrenal glands. *Am. J. Physiol.*, Vol. 100, No. 2, pp. 342-343.

ROUX, L. L. (1936). Sex physiology of sheep. *Onderstepoort J. Vet. Sci. and Anim. Industry*, Vol. 6, No. 2, pp. 465-717.

ROUX, L., AND F. E. HOFFMAN (1935). The management of rams. The ways in which rams may effect fertility in studs and flocks. *Farming in South Africa*, November, 1935.

RUSSEL, (1919). *Cit.*, Hammond (1932).

RUHL, A. (1925). Regelmasigkeit in Wechsel der Ovarialfunktion. *Arch. f. Gynakol.*, Vol. 124, pp. 1-26.

SCAMMON, R. E. (1922A). The height-weight index of the newborn infant. *Proc. Soc. Exp. Biol. Med.*, Vol. 20, pp. 102-104.

SCAMMON, R. E. (1922B). On the mode and transition from the fetal to the postnatal phase of growth in man. *Anat. Rec.*, Vol. 23, No. 1, pp. 34-35.

SCAMMON, R. E. (1937). Two simple nomographs for estimating the age and some of the major external dimensions of the human fetus. *Anat. Rec.*, Vol. 68, No. 2, pp 221-225.

SCAMMON, R. E., AND L. A. CALKINS (1923). Simple empirical formulae for expressing the lineal growth of the human fetus. *Proc. Soc. Exp. Biol Med.*, Vol. 20, pp. 353-356.

SCAMMON, R. E., AND H. L. DUNN (1922). Empirical formulae for the postnatal growth of the human brain and its major divisions. *Ibid.*, Vol. 20, pp. 114-117.

SCHNEIDER, H. (1936). Des gewichtsverhalten neugeborener Ferkel des veredelten Landschweines in den ersten Lebenstagen. *Inaug. Diss.*, Leipzig.

SCHOPF, O. (1935). Beitrag zum Bau der Hypophyse des Pferdes wahrend der Graviditat. *Wien. Tierarz. Monatschr.*, Vol. 23, p. 571.

SISSON, S. (1927). *The Anatomy of the Domestic Animals.* 2nd Edition. W. B. Saunders Co., Philadelphia and London.

SMETISKO, P. (1925). Untersuchungen uber das vorkommen von somatischen Geschlechtsmerkmalen bei Schweinen wahrend des Fotallebens, mit einer wissenschaftlichen Beilage. (Abstr.) *Jahresbericht Vet. Med.*, Vol. 45, p. 238.

SOMMER, M. (1912). Beitrag zur kenntnis der Involution des normalen Uterus des Rindes. *Zeitschr. f. Tiermed.*, Vol. 16, pp. 235-255.

STEGMANN, F. (1923). Messungen und Wagungen an Uterus des Schweines. *Inaug. Diss.*, Berlin.

STOTSENBERG, J. M. (1915). The growth of the fetus of the albino rat from the thirteenth to the twenty-second day of gestation. *Anat. Rec.*, Vol. 9, No. 1, pp. 667-682.

TRAUTMANN, D. (1917). Anatomisches und Histologisches uber die Cervix uteri. *Arch Wissensch. Prakt. Tierheilk.*, Vol. 43, pp. 239-261.

TURNER, C. W. (1930). The anatomy of the mammary gland of cattle, 1. Embryonic development. *Univ. Mo. Agric. Exp. Sta. Res. Bull.*, No. 160.

TURNER, C. W. (1934). The causes of the growth and function of the udder of cattle. *Univ. Mo. Agric. Exp. Sta. Bull.*, No. 339.

WARBRITTON, V., AND F. F. McKENZIE (1937). The pituitary glands of ewes in various phases of reproduction. *Univ. Mo. Agric. Exp. Sta. Res. Bull.*, No. 257.

WARWICK, B. L. (1926). Intra-uterine migration of ova in the sow. *Anat. Rec.*, Vol. 33, No. 1, pp. 29-33.

WILLIAMS, W. L. (1917). *Veterinary Obstetrics.* Published by the Author. Ithaca, N.Y.

WILLIAMS, W. L. (1921). *The Diseases of the Genital Organs of Domestic Animals.* Published by the Author, Ithaca, N.Y.

WOLFE, J. M., R. CLEVELAND, AND M. CAMPBELL (1933). Cyclic histological variations in the anterior hypophysis of the dog. *Zeitschr. f. Zellforsch. und Mikrosk. Anat.*, Vol. 17, No. 3, p. 420.

WOODMAN, H. E., AND J. HAMMOND (1923). The composition of secretions obtained from udders of heifers during pregnancy. *J. Agric. Sci.*, Vol. 13, No. 2, pp. 180-191.

WOODMAN, H. E., AND J. HAMMOND (1925). The mucous secretion of the cervix of the cow. *Ibid.*, Vol. 15, No. 2, pp. 107-124.

ZEIGER (1908). *Cit.*, Hammond (1927).

ZEITSCHMANN, O. (1923). *Lehrbuch der Entwicklungsgeschichte der Haustiere*, Part 1. Richard Schoetz, Berlin.

ZIMMERMANN, A. (1933). Uber die altersveranderungen der Schilddruse. (Abstr.) *Jahresbericht Vet. Med.*, Vol. 54, p. 419.

ZUNTZ (1909). *Cit.*, Hammond (1932).

APPENDIX "A"

Details of Experimental Animals.

Serial Number of Ewe.	D.O.B. Number of Ewe.	At Slaughter. Age Years.	At Slaughter. Body Wt. Kg.	At Slaughter. Brain Wt. Gm.	Previous Sexual History. Oestrum. 1936–37.	Previous Sexual History. Service. 1936–37.	Previous Sexual History. Parturition.	Experimental Treatment. Detect. of Oestr. Date.	Experimental Treatment. Detect. of Oestr. Hour.	Experimental Treatment. Slaughter. Date.	Experimental Treatment. Slaughter. Hour.	Foetus. Age in Days.
1	48110	4·00	36·50	126	✕ ✕	✕	—	19/7	9·30	4/8	14·00	15
2	44846	4·00	29·50	87	✕ ✕ ✕	—	—	14/7	9·30	31/7	9·00	16
3	29324	3·50	41·50	112	✕ ✕ ✕ ✕	✕	8/8/36	29/4	9·00	17/5	14·25	17
4	44496	3·50	36·50	110	✕ ✕	—	—	29/4	9·00	18/5	9·15	18
5	48117	3·50	36·50	101	✕ ✕	—	—	5/5	9·00	25/5	9·30	19
6	44811	3·75	28·00	95	—	—	—	13/4	10·00	4/5	9·15	20
7	39887	3·75	36·50	114	✕	—	—	13/4	10·00	5/5	9·15	21
8	44413	4·00	34·50	101	✕ ✕	✕	—	22/7	7·30	14/8	9·15	22
9	32668	4·00	36·00	100	✕ ✕ ✕ ✕	✕	2/11/36	15/7	9·00	10/8	9·20	25
10	41614	4·00	44·00	101	✕ ✕	—	—	19/7	9·30	16/8	9·15	27
11	44816	3·75	28·50	109	✕ ✕ ✕	✕	—	17/4	8·00	19/5	9·30	31
12	48131	3·75	28·00	97	✕ ✕	—	—	18/4	8·00	20/5	11·00	31
13	44444	3·50	35·50	102	✕	—	—	17/4	8·00	26/5	9·40	38
14	40632	3·75	34·00	100	✕	—	—	18/4	8·00	27/5	11·30	38
15	40663	3·50	33·00	108	✕	—	—	17/4	7·50	2/6	9·45	45
16	44347	3·75	32·00	97	✕ ✕ ✕	✕	—	18/4	8·00	3/6	11·30	45
17	44490	3·75	43·00	102	—	—	—	18/4	8·00	10/6	9·15	52
18	32490	3·75	34·00	104	✕ ✕ ✕	✕	—	17/4	7·50	9/6	9·45	52
19	38347	3·75	42·00	113	✕ ✕	✕	1/7/36	17/4	7·50	16/6	9·50	59
20	35711	4·00	36·50	100	✕ ✕	✕	—	18/4	8·00	17/6	11·40	59
21	44519	3·75	43·50	108	✕	✕	12/7/36	16/4	7·50	22/6	9·45	66
22	47664	4·00	43·50	110	✕	—	—	16/4	7·50	28/6	9·50	72
23	44759	3·75	37·50	101	✕	—	—	17/4	10·30	29/6	9·25	72
24	42642	4·25	34·02	102	✕ ✕ ✕ ✕	✕	6/1/37	15/7	9·00	5/10	9·10	81

Explanation :—

In connection with previous oestrum the number of observed occurences are indicated as follows—

✕ ✕ ✕ ✕ .... Very frequent.
✕ ✕ ✕ .... Less frequent.
✕ ✕ .... Occasional.
✕ .... Only one or two.

The dates and times in the 9th and 10th columns refer to the detection of the oestrum during which the ewe was successfully served. In these columns, and the following two, only the day and month are indicated as all this work was carried out during the course of one year, i.e. 1937.

APPENDIX "A".

DETAILS OF EXPERIMENTAL ANIMALS—(*continued*).

Serial Number of Ewe.	D.O.B. Number of Ewe.	At Slaughter.			Previous Sexual History.			Experimental Treatment.				Foetus.
		Age Years.	Body Wt. Kg.	Brain Wt. Gm.	Oestrum, 1936–37.	Service, 1936–37.	Parturition.	Detect. of Oestr. Date.	Detect. of Oestr. Hour.	Slaughter. Date.	Slaughter. Hour.	Age in Days.
25	44371	3·75	44·50	110	××	×	—	15/4	7·20	14/7	9·10	89
26	36165	3·50	45·00	106	××	×	—	14/4	8·30	13/7	9·15	89
27	44332	3·75	41·50	109	×××	—	—	15/4	10·30	20/7	9·50	95
28	42605	4·00	46·50	105	××	×	—	15/4	7·30	26/7	9·35	101
29	47666	3·75	30·50	102	×	—	—	15/4	7·50	27/7	9·20	102
30	44683	4·00	46·50	113	×	—	—	15/4	7·20	4/8	9·15	110
31	47665	4·00	36·50	105	×	—	—	14/4	8·50	3/8	9·15	110
32	32489	4·00	46·50	109	××	×	—	15/4	7·50	13/8	9·30	119
33	36084	4·00	48·00	109	××	—	—	13/4	8·30	11/8	9·25	119
34	44326	4·25	39·50	113	××	—	—	14/4	8·30	23/8	9·15	130
35	44408	4·00	40·00	96	××	—	—	15/4	10·30	24/8	9·15	130
36	38540	4·25	54·50	106	××××	×	—	13/4	7·15	1/9	9·25	140
37	48123	4·25	51·50	106	×	—	—	15/4	7·50	3/9	9·20	140
38	44338	4·00	46·50	102	×	—	—	15/4	10·30	10/9	9·05	147
39	44643	3·75	25·58	102	—	—	—	13/4	8·30	24/4	9·30	—
40	36417	3·75	38·10	116	×	×	—	9/5	9·00	22/5	9·15	—
41	44835	3·75	30·62	108	×	—	—	13/4	7·15	27/4	9·20	—
42	25948	4·25	30·84	94	××	×	1/7/36	14/7	9·30	7/8	9·00	—
43	44823	4·00	35·38	94	×	×	—	17/4	7·50	7/7	9·15	—
44	35719	4·00	34·47	111	×	×	24/6/36	15/7	9·00	12/8	9·00	—
45	48113	4·25	42·18	102	×××	×	—	15/7	9·00	20/9	9·10	—
46	44737	4·25	44·45	118	××××	×	—	22/7	7·30	12/10	9·35	—
47	47668	4·00	61·24	119	××	—	—	—	—	2/11	9·05	—
48	44401	4·25	36·29	103	××	—	—	—	—	3/11	9·00	—
49	44651	4·25	43·55	118	×××	—	—	—	—	4/11	9·00	—

Explanation :—

In connection with previous oestrum the number of observed occurences are indicated as follows—

×××× .... Very frequent.
××× .... Less frequent.
×× .... Occasional.
× .... Only one or two.

The dates and times in the 9th and 10th columns refer to the detection of the oestrum during which the ewe was successfully served. In these columns, and the following two, only the day and month are indicated as all this work was carried out during the course of one year, i.e. 1937.

APPENDIX "A".

Details of Genital Tract.

Serial Number of Ewe.	Weight in Grams.	Vagina. Dimensions.			Cervix. Dimensions.			Fallopian Tubes. Length.		Fallopian Tubes. Diameter.		Fallopian Tubes. Weight.		Body. Dimensions.		Wall of Cornua. Thickness.	
		L.	W.	T.	L.	W.	T.	P.	N.P.	P.	N.P.	P.	N.P.	L.	T.	P.	N.P.
1	22	10	3·2	0·30	4·0	2·1	0·30	16·5	16·0	0·20	0·20	0·50	0·50	1·5	0·40	0·40	0·40
2	18	10	3·5	0·20	5·0	1·5	0·35	17·0	15·0	0·15	0·15	0·30	0·30	2·5	0·40	0·40	0·40
3	35	13	3·7	0·40	7·0	2·5	0·40	15·0	15·5	0·20	0·20	0·70	0·75	2·5	0·50	0·40	0·40
4	17	11	3·5	0·30	5·0	2·5	0·40	17·0	15·0	0·15	0·15	0·50	0·60	2·0	0·40	0·40	0·40
5	17	10	3·0	0·30	5·0	2·0	0·25	15·0	15·5	0·15	0·15	0·45	0·40	1·5	0·25	0·25	0·25
6	17	10	2·7	0·40	4·5	2·5	0·40	16·0	15·6	0·30	0·30	0·50	0·50	2·0	0·40	0·40	0·40
7	35	11	4·0	0·40	4·8	3·0	0·40	18·5	18·5	0·20	0·20	0·65	0·70	3·0	0·40	0·40	0·40
8	22	12	4·5	0·25	6·0	1·5	0·20	17·5	17·0	0·20	0·20	0·40	0·50	1·5	0·40	0·40	0·40
9	26	10	3·5	0·40	6·0	1·8	0·40	15·0	15·0	0·20	0·20	0·48	0·40	2·0	0·40	0·40	0·40
10	15	12	3·2	0·20	6·0	1·5	0·30	16·0	15·0	0·15	0·20	0·45	0·40	1·8	0·40	0·40	0·40
11	26	13	3·5	0·25	4·5	3·0	0·40	15·6	15·0	0·20	0·20	0·50	0·60	2·5	0·40	0·40	0·40
12	17	11	2·4	0·25	5·0	1·5	0·35	11·5	13·5	0·20	0·20	0·40	0·40	2·0	0·30	0·25	0·25
13	13	11	2·8	0·35	6·5	1·5	0·40	19·0	20·0	0·20	0·20	0·60	0·70	2·5	0·30	0·30	0·30
14	22	12	3·2	0·30	5·5	1·8	0·30	16·5	18·0	0·15	0·15	0·45	0·45	3·0	0·20	0·20	0·25
15	22	12	2·6	0·35	6·5	1·7	0·40	18·0	18·0	0·20	0·20	0·70	0·70	2·5	0·40	0·40	0·40
16	10	10	2·2	0·25	5·5	1·6	0·35	18·0	19·0	0·20	0·20	0·50	0·50	2·5	0·40	0·40	0·40
17	22	11	2·8	0·25	5·5	1·6	0·30	17·0	18·0	0·20	0·20	0·60	0·60	3·5	0·25	0·30	0·25
18	23	13	3·5	0·20	5·5	1·6	0·40	18·5	18·0	0·20	0·20	0·60	0·55	2·5	0·25	0·25	0·25
19	22	14	3·5	0·30	6·5	1·8	0·30	16·0	17·0	0·20	0·20	0·45	0·45	5·0	0·25	0·25	0·25
20	17	11	3·0	0·30	6·0	1·8	0·30	14·0	16·0	0·20	0·20	0·45	0·45	4·0	0·30	0·30	0·30
21	18	14	3·0	0·30	7·0	1·5	0·20	17·0	18·5	0·20	0·20	0·50	0·50	4·5	0·25	0·20	0·25
22	22	15	3·5	0·20	7·0	2·0	0·20	17·0	17·5	0·20	0·20	0·50	0·50	5·0	0·20	0·20	0·20
23	25	11	4·0	0·20	6·0	2·0	0·30	14·5	15·0	0·20	0·20	0·50	0·50	6·0	0·30	0·30	0·30
24	32	14	5·0	0·20	5·5	1·5	0·30	16·0	15·5	0·15	0·15	0·25	0·25	7·5	0·25	0·25	0·25

N.B.—All measurements given in centimetres.

Abbreviations as follows:—

L. .... Length.
W. .... Width.
Wt. .... Weight.
T. .... Thickness.
P. .... Pregnant Side.
N.P. .... Non-pregnant Side.

In the case of Ewes No's 39 to 49 (i.e. the non-pregnant group) the left side measurements are tabulated under "P" (pregnant side at 0 days) while the measurements on the right hand side are regarded as those of the non-pregnant side at 0 days, and are given under "N.P.".

APPENDIX "A"

Details of Genital Tract—(*continued*).

Serial Number of Ewe.	Weight in Grams.	Vagina.			Cervix.			Fallopian Tubes.						Body.		Wall of Cornua.	
		Dimensions.			Dimensions.			Length.		Diameter.		Weight.		Dimensions.		Thickness.	
		L.	W.	T.	L.	W.	T.	P.	N.P.	P.	N.P.	P.	N.P.	L.	T.	P.	N.P.
25	28	13	5·0	0·35	8·5	2·5	0·30	18·0	17·5	0·20	0·20	0·45	0·45	5·0	0·20	0·20	0·20
26	24	13	3·5	0·25	7·2	2·0	0·40	20·0	19·0	0·20	0·20	0·65	0·57	5·0	0·20	0·15	0·20
27	18	15	3·5	0·15	7·0	1·8	0·30	19·0	18·5	0·15	0·15	0·40	0·30	7·0	0·20	0·20	0·20
28	23	13	3·2	0·25	7·5	2·0	0·30	18·0	18·5	0·20	0·20	0·65	0·65	6·0	0·25	0·25	0·25
29	23	13	4·0	0·20	7·5	2·5	0·30	16·0	16·0	0·20	0·20	0·50	0·50	5·0	0·20	0·15	0·20
30	29	15	4·0	0·20	8·0	2·4	0·40	20·0	19·0	0·20	0·20	0·62	0·62	5·5	0·25	0·15	0·15
31	36	12	4·5	0·20	8·0	2·3	0·30	17·0	18·0	0·15	0·15	0·50	0·50	6·5	0·20	0·15	0·15
32	34	14	7·0	0·15	9·0	2·5	0·30	14·5	15·5	0·20	0·20	0·40	0·40	7·0	0·15	0·15	0·15
33	28	13	4·0	0·25	8·5	2·5	0·40	18·5	18·0	0·20	0·20	0·60	0·60	10·0	0·15	0·15	p·15
34	33	18	6·0	0·15	9·0	2·6	0·50	17·5	18·0	0·20	0·20	0·50	0·50	6·0	0·20	0·15	0·15
35	29	14	5·5	0·15	8·5	2·3	0·40	16·0	18·0	0·20	0·20	0·40	0·50	10·5	0·25	0·15	0·15
36	32	16	6·0	0·20	8·0	3·0	0·55	19·0	20·5	0·20	0·20	0·40	0·50	8·0	0·20	0·15	0·15
37	43	14	7·0	0·20	8·5	3·0	0·60	19·0	18·5	0·20	0·20	0·60	0·50	7·5	0·20	0·15	0·15
38	39	16	7·5	0·15	10·0	4·0	0·70	18·0	18·0	0·20	0·20	0·60	0·60	11·5	0·50	0·30	0·50
39	30	10	3·0	0·30	4·0	2·0	0·30	13·0	13·0	0·20	0·20	0·60	0·50	2·0	0·50	0·50	0·50
40	29	12	3·2	0·30	6·0	1·3	0·35	16·0	15·0	0·30	0·25	1·10	0·90	2·5	0·30	0·30	0·30
41	20	10	3·0	0·20	4·0	2·0	0·30	15·0	16·0	0·20	0·20	0·75	0·70	2·0	0·40	0·30	0·30
42	18	10	3·5	0·20	5·5	1·5	0·30	14·0	12·0	0·20	0·20	0·40	0·40	1·5	0·40	0·40	0·40
43	16	11	3·0	0·20	5·0	1·8	0·30	13·5	14·0	0·20	0·20	0·50	0·40	2·5	0·30	0·50	0·50
44	28	11	4·5	0·30	4·5	2·5	0·40	14·0	13·0	0·20	0·20	0·40	0·40	3·0	0·50	0·50	0·60
45	23	13	4·8	0·15	6·5	1·8	0·30	19·0	18·5	0·20	0·20	0·60	0·50	3·0	0·40	0·40	0·40
46	24	10	4·0	0·40	6·0	1·5	0·30	15·5	16·5	0·20	0·20	0·30	0·35	2·0	0·60	0·60	0·60
47	24	11	3·3	0·50	5·5	1·6	0·70	18·0	17·5	0·20	0·20	0·50	0·50	2·0	0·50	0·50	0·50
48	18	10	3·5	0·60	4·5	1·7	0·50	16·0	15·5	0·20	0·20	0·30	0·30	1·5	0·50	0·50	0·50
49	17	10	2·4	0·50	5·0	1·5	0·50	15·5	14·5	0·20	0·20	0·30	0·30	1·8	0·50	0·50	0·50

N.B.—All measurements given in centimetres.

Abbreviations as follows:—

L. .... Length.
W .... Width.
Wt. .... Weight.
T. .... Thickness.
P. .... Pregnant Side.
N.P. .... Non-pregnant Side.

In the case of Ewes No's 39 to 49 (i.e. the non-pregnant group) the left side measurements are tabulated under "P" (pregnant side at 0 days) while the measurements on the right hand side are regarded as those of the non-pregnant side at 0 days, and are given under "N.P.".

APPENDIX "A".

Details of Genital Tract—(*continued*).

Serial Number of Ewe.	Uterus. Weights in Grams. D.	E.	W.	Greater Curv. P.	N.P.	Lesser Curv. P.	N.P.	Circumference. P.	N.P.	Plac. Weight in Gram.	Cotyledons. Number. P.	N.P.	Diameter. P.	N.P.	Height. P.	N.P.
1	45	44	—	19·0	18·0	12·5	13·0	5·0	5·0	—	36	37	0·40	0·40	0·15	0·15
2	37	36	—	21·0	18·0	16·0	13·0	5·0	5·0	—	51	45	0·40	0·40	0·15	0·15
3	60	58	—	16·0	15·5	14·5	14·0	5·0	5·0	—	47	45	0·30	0·30	0·10	0·10
4	27	25	—	15·5	14·0	14·0	13·0	5·2	5·0	—	45	45	0·30	0·30	0·15	0·15
5	31	27		16·0	15·5	14·0	15·0	5·2	4·3	—	49	48	0·30	0·30	0·10	0·10
6	23	14	—	16·0	15·0	14·0	13·0	5·2	4·4	—	36	35	0·40	0·40	0·15	0·15
7	53	39	—	17·5	16·0	15·0	14·0	6·0	4·8	—	41	39	0·40	0·40	0·15	0·15
8	47	37	—	17·5	18·0	11·0	13·0	7·0	4·5	—	44	44	0·40	0·40	0·15	0·15
9	72	51	—	19·0	18·0	11·0	12·5	7·5	6·0	—	43	44	0·40	0·40	0·10	0·10
10	91	39	—	26·0	22·0	15·0	13·0	9·0	7·0	—	49	49	0·50	0·40	0·20	0·15
11	163	59	—	21·0	18·5	16·0	17·0	11·5	9·0	—	38	37	0·60	0·50	0·20	0·20
12	142	47	—	25·0	22·0	14·5	13·0	10·8	8·7	—	51	47	0·60	0·50	0·30	0·30
13	134	58	—	23·0	21·0	14·5	16·5	11·0	8·0	—	45	38	1·00	0·80	0·30	0·30
14	173	60	—	28·0	24·0	16·5	15·0	11·0	9·5	—	30	32	0·90	0·70	0·30	0·30
15	350	123	—	36·0	35·0	21·5	19·0	13·5	11·0	—	41	36	1·75	0·85	0·50	0·40
16	388	116	—	37·0	36·0	24·0	22·0	16·0	11·5	—	42	35	1·00	0·70	0·40	0·30
17	635	305	—	40·0	31·0	21·0	23·0	23·5	17·0	—	47	40	2·50	2·20	0·80	0·70
18	625	200	—	50·5	45·0	25·0	30·0	30·0	20·0	—	33	42	2·00	1·75	0·60	0·50
19	1,100	473	149	46·0	48·0	32·0	29·0	24·0	20·0	324	47	46	2·75	2·50	1·00	0·90
20	753	287	119	44·0	34·0	28·0	25·5	21·5	15·0	168	42	32	3·00	2·25	1·10	1·00
21	1,545	735	190	60·0	46·0	27·0	29·0	29·5	18·0	545	53	45	4·00	3·50	1·30	1·00
22	3,124	1,120	275	74·0	65·0	38·0	50·0	34·0	25·0	845	49	42	4·00	3·50	1·40	1·40
23	1,488	567	165	50·0	52·0	30·0	24·0	24·0	27·0	402	33	32	3·00	3·00	1·60	1·60
24	1,932	627	195	59·0	43·0	30·0	31·0	33·0	22·0	432	38	32	3·25	3·00	1·50	1·50

Abbreviations as follows:—

D. . . . . Dressed Uterus.
E. . . . . Empty Uterus.
W. . . . . Uterine Wall (Empty Uterus less Placenta).
Plac. . . . . Placenta.
P. and N.P. . . . . As explained previously.

Non-pregnant Group treated as indicated on previous page.

APPENDIX "A".

Details of Genital Tract—(*continued*).

Serial Number of Ewe.	Uterus.									Plac.	Cotyledons.					
	Weights in Grams.			Greater Curv.		Lesser Curv.		Circumference.		Weight in Gram.	Number.		Diameter.		Height.	
	D.	E.	W.	P.	N.P.	P.	N.P.	P.	N.P.		P.	N.P.	P.	N.P.	P.	N.P.
25............	2,281	700	248	56·0	46·0	32·0	27·0	30·0	29·0	452	45	50	3·25	3·25	1·50	1·50
26............	2,402	692	252	77·0	49·0	42·0	36·0	36·0	17·0	440	45	35	3·20	3·20	1·20	1·20
27............	2,750	826	230	71·0	60·0	34·0	28·0	34·0	25·0	596	40	40	3·75	3·50	1·75	1·60
28............	2,640	682	255	70·0	48·0	31·0	28·0	41·0	22·0	427	42	36	3·75	3·50	1·75	1·60
29............	2,600	474	187	57·0	41·0	36·0	28·0	37·0	21·0	287	38	33	3·25	3·10	1·50	1·50
30............	3,521	685	309	81·0	60·0	38·0	40·0	40·0	22·0	376	46	39	2·75	2·75	1·40	1·40
31............	2,675	480	238	72·0	55·0	36·0	32·0	34·0	20·0	242	25	27	3·00	3·00	1·30	1·30
32............	3,950	570	260	89·0	59·0	43·0	34·0	40·0	24·0	310	41	45	2·50	2·50	1·30	1·30
33............	3,050	465	260	84·0	59·0	40·0	32·0	37·0	20·0	205	32	36	2·25	2·25	1·50	1·50
34............	4,448	467	387	82·0	62·0	54·0	39·0	43·0	23·0	280	45	37	3·00	3·00	1·50	1·50
35............	5,296	680	342	108·0	66·0	43·0	40·0	45·0	22·0	338	41	42	3·00	3·00	1·40	1·40
36............	6,728	870	480	118·0	72·0	40·0	43·0	40·0	20·0	390	51	51	3·00	3·00	1·00	1·00
37............	6,512	860	523	97·0	58·0	47·0	44·0	52·0	24·0	337	39	35	3·00	3·00	1·10	1·10
38............	6,420	667	477	95·0	55·0	35·0	33·0	47·0	27·0	190	39	39	3·00	3·00	0·80	0·80
39............	32	32	—	17·0	18·0	13·0	14·0	4·5	4·5	—	48	48	0·20	0·20	0·10	0·10
40............	71	71	—	16·0	15·5	13·0	12·0	6·0	5·5	—	58	55	0·25	0·25	0·20	0·20
41............	30	30	—	16·0	15·0	11·0	10·5	5·0	5·0	—	46	46	0·30	0·30	0·10	0·10
42............	49	49	—	17·0	16·0	12·5	12·5	5·5	6·0	—	44	45	0·20	0·20	0·15	0·15
43............	30	30	—	14·5	14·0	10·0	9·5	4·0	4·5	—	40	42	0·20	0·20	0·15	0·15
44............	64	64	—	14·0	14·5	11·5	9·5	6·5	7·5	—	47	41	0·30	0·30	0·15	0·15
45............	38	38	—	14·5	15·0	11·0	11·0	5·0	5·0	—	47	44	0·30	0·30	0·20	0·20
46............	53	53	—	20·0	17·0	14·5	14·0	5·0	5·0	—	44	40	0·40	0·40	0·20	0·20
47............	54	54	—	18·0	18·0	13·0	12·0	5·5	5·5	—	44	40	0·30	0·40	0·15	0·20
48............	22	22	—	11·0	11·0	9·0	9·0	3·5	4·0	—	44	46	0·30	0·30	0·10	0·10
49............	27	27	—	13·5	13·0	9·0	9·0	5·0	5·0	—	51	51	0·30	0·30	0·20	0·20

Abbreviations as follows:—

D.. Dressed Uterus.
E.. Empty Uterus.
E.. Empty Uterus.
W.. Uterine Wall (Empty Uterus less Placenta).
Plac.................................... Placenta.
P. and N.P.......................... As explained previously.

Non-pregnant Group treated as indicated on previous page.

APPENDIX "A".

Foetal Fluids and Membranes.

Serial Number of Ewe.	Foetal Memb.		Foetal Fluids.							
	Weight in Grams.	Volume in C. cm.	Volume in C. cm.		Spec. Gravity.		Hydr. Ion. Conc.		Appearance.	
			All.	Amn.	All.	Amn.	All.	Amn.	Allantoic.	Amniotic.
1	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—
3	—	—	0·5	—	—	—	—	—	Clear, colourless and watery.	—
4	0·3	0·3	1·0	—	—	—	—	—	Clear, colourless and watery.	—
5	0·4	0·3	2·0	—	—	—	8·2	—	Clear, colourless and watery.	—
6	—	—	3·0	0·5	—		—	—	Clear, colourless adn watery.	Clear, colourless, watery.
7	—	—	12·0	0·5	—	—	—	—	Clear, colourless and watery.	Clear, colourless, watery.
8	0·8	0·8	7·5	0·5	—	—	7·6	—	Clear, colourless and watery.	Clear, colourless, watery.
9	3·8	3·6	14·0	0·5	—	—	7·4	—	Clear, colourless and watery.	Clear, colourless, watery.
10	6·0	5·8	37·0	2·0	—		7·6	—	Clear, colourless and watery.	Clear, colourless, watery.
11	18·9	15·8	72·0	2·5	1·003		8·4	8·6	Clear, colourless and watery.	Clear, colourless, watery.
12	18·4	16·3	69·0	4·0	1·003	—	8·0	8·4	Clear, colourless and watery.	Clear, colourless, watery.
13	31·0	16·0	26·0	19·0	—	—	8·0	8·0	Clear, colourless and watery.	Clear ,colourless, watery.
14	23·8	14·5	68·0	15·5	1·003	—	7·8	8·0	Clear, colourless and watery.	Clear, colourless, watery.
15	56·7	56·0	92·0	57·0	1·009	1·004	7·2	7·4	Clear, colourless and watery.	Clear, colourless, watery.
16	58·0	54·7	115·0	72·0	1·005	1·005	7·2	7·4	Slightly cloudy............	Clear, colourless, watery.
17	47·5	46·5	65·0	185·0	1·007	1·003	7·0	7·2	Pale lemon tinge..........	Clear, colourless, watery.
18	92·0	86·0	92·0	152·0	1·008	1·002	7·2	7·4	Pale lemon tinge..........	Pale lemon tinge.
19	93·0	92·5	174·0	203·0	1·010	1·004	6·8	7·1	Pale lemon tinge..........	Pale lemon tinge.
20	83·6	79·0	117·0	198·0	1·006	1·009	7·1	7·0	Slightly viscid............	More distinct lemon.
21	80·5	78·0	106·0	480·0	1·016	1·006	7·0	7·1	Lemon colour; turbid......	Bright amber colour.

Abbreviations as follows:—

All.................................. Allantoic fluid.
Amn................................ Amniotic fluid.
Hydr.-Ion Conc.................. Hydrogen-Ion Concentration.
Memb.............................. Membranes.

APPENDIX "A".

Foetal Fluids and Membranes—(*continued*).

Serial Number of Ewe.	Foetal Memb.		Foetal Fluids.							
	Weight in Grams.	Volume in C. cm.	Volume in C. cm.		Spec. Gravity.		Hydr. Ion. Conc.		Appearance.	
			All.	Amn.	All.	Amn.	All.	Amn.	Allantoic.	Amniotic.
22	330·0	300·0	600·0	500·0	1·005	1·002	7·0	7·1	Lemon colour; turbid......	Less distinct amber.
23	50·0	46·0	—	—	—	—	—	—	—	—
24	80·0	79·0	40·0	750·0	1·015	1·004	6·8	7·2	Lemon colour; turbid......	Lemon colour.
25	85·0	82·0	65·0	820·0	1·013	1·002	6·8	7·3	Lemon changing to amber..	Slightly viscid.
26	88·0	85·0	115·0	940·0	1·015	1·007	7·2	7·2	Amber; fairly turbid.......	Amber; more viscid.
27	122·0	120·0	71·0	750·0	1·016	1·011	7·2	7·4	Dark amber; more viscid..	Amber; more viscid.
28	110·0	105·0	215·0	480·0	1·016	1·066	6·6	6·8	Dark amber; more viscid..	Amber; more viscid.
29	112·0	110·0	390·0	590·0	1·010	1·004	7·2	7·0	Dark amber; more viscid..	Lemon; fairly cloudy.
30	155·0	150·0	535·0	550·0	1·011	1·003	6·6	7·0	Dark amber; more viscid..	Lemon fairly cloudy.
31	139·0	136·0	370·0	360·0	1·012	1·003	7·0	7·2	Dark amber: less cloudy...	Pale lemon; very cloudy.
32	147·0	140·0	500·0	620·0	1·013	1·003	6·6	7·4	Dark amber; watery.......	Deeper shade lemon.
33	115·0	113·0	350·0	330·0	1·011	1·000	6·6	7·2	Dark amber; watery.......	Deeper shade lemon; very viscid.
34	162·0	158·0	725·0	295·0	1·010	1·002	6·6	7·4	Dark amber: watery.......	Deeper shade lemon; very viscid.
35	225·0	220·0	685·0	275·0	1·011	1·004	7·0	7·0	Dark amber; not turbid...	Pale lemon; creamy.
36	230·0	225·0	770·0	375·0	1·012	1·003	6·9	6·8	Dark amber: not turbid...	Pale lemon; less viscid.
37	185·0	180·0	550·0	350·0	1·015	1·002	6·6	6·8	Dark amber; not turbid...	Pale lemon; less viscid.
38	162·0	160·0	1,080·0	350·0	1·009	1·002	7·2	7·4	Distinct amber colour, almost clear and watery	Very pale lemon, cloudy and fairly viscid.

Abbreviations as follows:—

All.......................... .... Allantoic fluid.
Amn.......................... Amniotic fluid.
Hydr.-Ion Conc.................. Hydrogen-Ion Concentration.
Memb.......................... Membranes.

APPENDIX "A".

Endocrines, Ovaries and Mammary Gland.

Serial Number of Ewe.	Hypophysis.				Ovaries.								Pineal.	Adrenal.	Thyroid.	Mam Gld
	Weight Gram.	Dimensions.			Length.		Width.		Depth.		Weight.		Weight in Gram.	Combd. Weight.	Weight in Gram.	Weight in Gram.
		L.	W.	D.	C.L.	N.C.L.	C.L.	N.C.L.	C.L.	N.C.L.	C.L.	N.C.L.				
1	0·60	1·4	1·1	0·8	1·9	1·5	1·5	0·9	1·2	0·9	1·6	1·0	0·10	3·10	2·60	182
2	0·55	1·2	1·0	0·8	1·3	1·0	1·3	0·9	1·0	0·8	1·0	0·3	0·10	3·00	2·00	122
3	—	—	—	—	1·9	1·3	1·6	1·3	1·3	0·8	1·7	0·9	—	—	—	110
4	0·75	1·4	1·0	0·8	1·8	1·3	1·5	1·1	0·9	0·6	1·2	0·6	—	—	—	72
5	0·60	1·3	1·1	0·8	1·6	1·0	1·1	0·9	1·0	0·7	1·2	0·6	—	—	—	—
6	0·60	1·4	1·1	0·5	1·6	1·2	1·3	1·0	0·9	0·6	1·1	0·5	—	—	—	33
7	0·55	1·5	1·0	0·6	1·3	1·3	1·3	1·1	1·3	0·8	1·2	0·7	—	—	—	93
8	1·00	1·3	1·0	1·0	1·8	1·1	1·1	1·0	0·9	1·0	1·1	0·7	0·10	3·30	2·30	124
9	0·60	1·4	1·0	0·8	1·5	1·6	1·2	1·2	1·2	0·6	1·3	0·7	0·10	3·00	2·50	168
10	0·60	1·3	1·1	0·8	1·3	1·3	1·4	0·9	1·0	0·9	2·0	0·7	0·20	0·60	0·50	160
11	0·80	1·5	1·1	0·6	1·8	1·5	1·5	1·1	1·1	0·6	1·6	0·7	—	—	—	—
12	0·70	1·4	1·0	0·9	1·7	1·3	1·2	0·9	1·0	0·6	1·3	0·5	—	—	—	62
13	0·85	1·4	0·9	0·9	1·6	1·1	1·2	1·1	1·1	0·8	1·4	0·7	—	—	—	155
14	0·60	1·3	1·0	0·8	1·6	1·5	1·2	0·9	1·1	0·7	1·3	0·7	—	—	—	167
15	0·60	1·3	1·0	0·8	1·7	1·6	1·4	1·1	0·9	0·7	1·2	0·7	—	—	—	157
16	0·60	1·2	1·0	0·8	2·0	1·5	1·3	0·9	0·9	0·8	1·6	0·8	—	—	—	134
17	1·00	1·7	1·1	1·0	1·8	1·5	1·5	1·1	0·9	0·5	1·7	0·7	—	—	—	212
18	0·80	1·3	1·2	0·8	1·7	1·5	1·2	0·8	1·0	0·6	1·4	0·5	—	—	—	279
29	0·80	1·5	1·0	0·9	1·5	1·6	1·4	1·2	1·0	0·8	1·4	1·1	—	—	—	187
20	0·70	1·4	1·1	0·8	1·8	1·5	1·3	0·9	1·0	0·8	1·5	0·6	—	—	—	150
21	—	—	—	—	1·8	1·7	1·4	1·0	0·8	0·4	1·1	0·6	—	—	—	195
22	0·95	1·5	1·2	0·8	1·8	1·6	1·3	0·9	1·0	0·5	1·6	0·7	—	—	—	237
23	0·60	1·5	1·1	0·8	1·8	1·2	1·2	1·0	0·8	0·6	1·4	0·6	—	—	—	175
24	0·70	1·4	0·9	0·8	1·6	1·0	1·4	0·9	0·9	0·6	1·2	0·5	0·11	2·70	1·80	150
25	0·90	1·4	1·2	0·8	1·9	1·7	1·5	1·0	1·0	0·6	1·5	0·9	0·20	3·70	2·50	183

Abbreviations as follows: —

L. .... Length.
W. .... Width.
D. .... Depth.
C.L. .... With Corpus Luteum.
N.C.L. .... Without Corpus Luteum.
Mam. Gld .... Mammary Gland.

APPENDIX "A".

Endocrines, Ovaries and Mammary Gland—(*continued*).

Serial Number of Ewe.	Hypophysis.				Ovaries.								Pineal.	Adrenal	Thyroid	Mam Gld
	Weight Gram.	Dimensions.			Length.		Width.		Depth.		Weight.		Weight in Gram.	Combd. Weight.	Weight in Gram.	Weight in Gram.
		L.	W.	D.	C.L.	N.C.L.	C.L.	N.C.L.	C.L.	N.C.L.	C.L.	N.C.L.				
26	0·70	1·5	1·1	0·7	1·7	1·7	1·3	1·1	0·9	0·5	1·2	0·7	0·20	4·05	1·60	280
27	0 80	1·3	1·2	0·9	1·5	1·5	1·4	0·6	0·9	0·4	1·2	0·5	0·14	3·50	2·50	137
28	0·90	1·4	1·0	1·0	1·8	1·5	1·3	0·9	1·0	0·5	1·3	0·6	0·20	3·80	3·10	294
29	0·55	1·4	0·8	0·9	1·4	1·4	1·2	1·1	1·0	0·5	1·0	0·7	0·10	3·60	3·40	165
30	0·80	1·5	1·1	0·9	1·6	1·4	1·2	1·0	1·0	0·5	1·3	0·6	0·10	3·00	1·60	397
31	0·65	1·4	1·0	0·8	2·0	1·7	1·3	0·9	1·0	0·7	1·2	0·7	0·15	2·30	2·20	295
32	0·60	1·3	1·1	0·8	1·6	1·7	1·1	0·9	0·9	0·5	1·0	0·6	0·15	2·10	0·90	340
33	0·80	1·5	1·0	0·9	1·6	1·3	1·1	1·0	0·9	0·5	1·0	0·6	0·20	3·70	1·80	575
34	0·80	1·4	1·1	0·8	1·5	1·3	1·0	0·9	0·7	0·6	0·7	0·5	0·10	3·00	1·60	365
35	0·80	1·4	0·9	1·0	1·3	1·2	1·1	0·8	1·0	0·6	1·0	0·4	0·11	5·40	3·00	360
36	1·00	1·5	1·0	1·0	1·5	1·5	1·3	1·1	0·9	0·4	0·8	0·4	0·20	4·70	2·90	1,120
37	1·40	1·6	1·3	1·1	2·0	1·7	1·1	1·2	0·9	0·8	1·3	0·9	0·10	3·70	3·70	1,285
38	0·80	1·4	0·9	1·0	1·9	1·5	1·3	1·0	0·6	0·5	1·2	0·7	0·15	3·20	2·30	900
39	0·75	1·3	1·0	0·6	1·5	1·0	1·2	0·9	0·9	0·7	1·1	0·5	—	—	—	80
40	0·60	1·6	0·9	0·7	1·8	1·0	1·1	0·9	0·8	0·5	1·2	0·5	—	—	—	90
41	0·60	—	—	—	1·8	1·4	1·0	1·2	0·8	0·7	1·0	0·8	—	—	—	156
42	—	—	—	—	1·3	1·2	1·2	1·0	1·0	0·5	1·2	0·5	0·10	2·50	3·00	—
43	0·55	1·2	1·0	0·8	1·7	1·2	1·1	1·0	0·9	0·7	1·4	0·6	—	—	—	154
44	1·00	1·4	1·2	0·9	1·7	1·4	1·0	1·0	0·9	0·6	1·0	0·4	0·10	4·50	3·20	254
45	0·70	1·4	1·0	0·8	1·8	1·1	1·3	1·0	1·0	0·6	1·6	0·6	0·15	2·50	3·00	203
46	1·10	1·6	1·2	1·0	1·5	1·3	1·3	1·3	1·5	0·6	2·5	0·8	0·10	2·90	2·00	275
47	1·20	1·8	1·1	1·0	1·6	1·7	1·3	1·2	1·2	1·2	1·6	1·5	0·10	3·80	2·90	351
48	0·60	1·3	1·0	0·8	1·3	1·2	0·9	0·8	0·9	0·6	0·7	0·5	0·10	2·00	2·10	184
49	0·65	1·3	0·9	0·8	1·2	1·2	1·0	1·0	0·8	0·7	0·6	0·6	0·15	3·30	1·60	252

In the case of Ewes No. 48 and 49 no Corpus Luteum was present. In these instances the left ovary is tabulated under C.L. and the right under N.C.L.

Abbreviations as follows:—

L. .... Length.
W. .... Width.
D. .... Depth.
C.L. .... With Corpus Luteum.
N.C.L. .... Without Corpus Luteum.
Mam. Gld .... Mammary Gland.

APPENDIX "A".

Dimensions of Foetus.

Serial Number of Ewe.	Age of Foetus Days.	Sex of Foetus.	Weight in Grams.	Crown Rump Str.	Crown Rump Curv.	Back Line.	Tail Length.	Length of Head.	Width of Head.	Heart Girth.	Length of Trunk.	Length of Forelimb.	Length of Hindlimb	Length Vertb. Column.
4	18	—	0·05	0·4	1·0	—	—	—	—	—	—	—	—	—
5	19	—	0·10	0·5	1·4	—	—	—	—	—	—	—	—	—
6	20	—	0·07	0·7	1·8	—	—		—	—	—	—	—	—
7	21	—	—	—	—	—	—	—	—	—	—	—	—	—
8	22	—	0·15	0·8	1·6	—	—	—	—	—	—	—	—	—
9	25	—	0·30	1·4	3·8	—	—	—	—	—	—	—	—	—
10	27	—	0·65	1·6	3·7	—	—	—	—	—	—	—	—	—
11	31	—	1·10	2·0	4·4	—	—	—	—		—	—	—	—
12	31	—	1·00	2·0	4·5	—	—	—	—	—	—	—	—	—
13	38	—	4·7	3·5	6·9	4·1	1·5	1·6	0·9	4·0	1·9	1·9	1·8	5·6
14	38	—	4·1	3·4	6·5	3·8	1·2	1·6	1·0	3·4	1·9	1·8	1·7	5·0
15	45	—	9·7	5·0	8·9	5·3	1·9	2·1	1·3	4·5	3·2	3·0	3·1	7·2
16	45	—	12·1	5·7	9·3	5·0	2·3	3·4	1·4	5·0	3·2	3·3	3·2	7·3
17	52	F.	27·3	8·5	12·0	7·9	2·0	3·2	1·9	7·1	4·5	4·9	4·9	10·6
18	52	M.	29·6	9·0	12·7	7·8	2·9	3·2	2·0	7·3	4·5	4·9	5·0	10·8
19	59	F.	54·3	11·0	13·2	9·6	3·1	3·9	2·2	9·0	6·6	6·3	6·9	12·7
20	59	F.	39·2	10·2	13·3	8·8	3·8	3·8	2·1	8·1	5·7	5·5	5·8	12·6
21	66	F.	96·6	13·5	16·0	11·3	4·2	4·9	2·7	10·8	8·0	7·9	8·1	15·5
22	72	F.	161	16·5	21·0	14·5	5·8	5·8	3·1	12·3	10·2	10·2	11·1	20·3
23	72	F.	152	16·2	20·0	13·9	6·3	5·8	3·3	12·8	9·8	10·2	10·9	20·2
24	81	M.	427	20·0	23·5	16·9	7·3	7·0	3·8	16·5	12·8	12·5	13·5	24·2
25	89	F.	485	22·5	28·0	19·0	7·8	8·3	4·4	17·5	14·0	15·6	17·6	26·8
26	89	F.	470	22·5	28·5	19·7	7·3	7·9	4·1	17·3	14·0	15·1	16·8	27·0
27	95	M.	737	28·0	32·5	21·2	10·2	9·2	5·0	21·7	17·5	17·7	20·0	31·4
28	101	F.	970	31·5	35·5	27·9	10·3	10·0	5·1	22·6	19·4	21·2	24·7	38·2
29	102	M.	947	29·0	35·0	24·1	10·9	9·8	5·1	22·5	19·1	19·9	22·5	35·0
30	110	M.	1,460	33·0	38·2	30·5	11·3	11·1	5·5	25·5	22·5	22·6	29·0	41·8
31	110	M.	1,205	33·5	39·0	28·3	12·5	11·1	5·4	25·2	21·5	23·3	26·8	40·8
32	120	F.	1,875	38·5	43·0	31·7	16·9	11·9	5·9	29·0	24·5	30·3	34·4	48·6
33	120	F.	1,692	34·5	41·0	32·0	14·6	10·3	5·6	26·8	22·5	27·3	32·0	46·6
34	130	M.	2,310	40·0	47·0	34·0	17·0	12·0	6·1	29·0	25·0	31·0	38·1	51·0
35	130	M.	3,120	42·5	48·0	37·0	17·0	13·0	6·8	32·6	29·9	34·5	41·4	54·0
36	140	M.	4,120	47·0	54·0	41·8	17·2	13·1	7·2	36·0	29·6	40·8	47·4	59·0
37	140	M.	4,120	45·0	54·0	42·9	17·1	13·2	7·2	36·2	31·0	38·3	45·7	60·0
38	147	F.	3,800	44·0	51·5	42·0	17·0	13·0	6·7	34·0	31·0	39·1	44·0	59·0

All length measurements given in centimeteres.

APPENDIX " B ".

Comments on the Methods Adopted in Fitting Curves.

As indicated in the text the weight-age data were the first to be considered. The individual observations were translated to logarithmic form. For convenience let x siginfy these values of the age data, and y those of the weight data. It appeared that the plotting of y against x resulted in a straight-line distribution. Consequently the regression coefficient b was determined and a straight line with the formula $Y = (\bar{y} - b\bar{x}) + bx$ was fitted.

Next the standard deviation of a single observation about this line was calculated. Let this be S.

The coefficient of variation = 100 × S %

The significance of the coefficient of regression was tested by calculating

$$t = \frac{6}{SE6}$$

Although the significance was high the fact that the coefficient of variation was large and that the actual observations were badly scattered about, the regression line led one to believe that a better " fit " would be obtained by introducing also the square of the age, i.e., fitting a parabola. For this the formula is:—

$$y = \bar{y} + b_1 (x_1 - \bar{x}_1) + b_2 (x_2 - \bar{x}_2)$$

Here x_2 represents the square of the age, and b_1 and b_2 are the coefficients. The former signifies the tendency towards straightness while the latter introduces the curved tendency.

The coefficient of variation is calculated as before, while the significance of both coefficients is tested in the same manner as previously described.

Both on account of the lower coefficient of variation and the improved distribution of the data about the line, it is concluded that this line " fits " the data more satisfactorily than the previous one.

It will be remembered that in the above calculation both age and weight were represented in their logarithmic forms. In order to eliminate these logarithms it is necessary to take antilogarithms on both sides of the question. In this way it is possible to determine for any series of age values the corresponding expected values of weight. The construction of a smooth weight-age curve is then a simple matter. All the curves presented in chapter 4 (in the section concerning the foetus) were constructed in this manner.

In the majority of cases it was obvious from the plotting of the logarithms of the dimensions against those of age that a straight line would not give the best " fit ". Therefore, this equation was not calculated. However, in all instances the coefficient of variation of the linear logarithmic trend was determined. This was done in order to establish definitely, in each case, the superiority of the curved logarithmic trend.

It is to be noted that the technique employed in these calculations, and, in fect, throughout the whole work, is that standardized by R. A. Fisher in his " Statistical Methods for Research Workers ", from which all basic formulae have been obtained.

AUTHOR INDEX.

Volume 13, Nos. 1 and 2.

SUBJECT INDEX.

Volume 13, Nos. 1 and 2.

TABLE 6.—*Absorbed Group Sera.*

ANTIGENS.	UNOBSERVED GROUP SERA.					ONDERSTEPOORT, GROUP SERUM ABSORBED BY.												ABSORBED SERA.			
	Onderstepoort.	*Reading.*	*Newport.*	*Binns.*	*Kunzendorf.*	*Binns.*	*London.*	*Binns+London.*	*Binns+London+Kunzendorf.*	*Kunzendorf.*	*Paratyphi C.*	*Reading.*	*Newport.*	*Sendai.*	*Anatum.*	*Anatum+Binns.*	*Anatum+Binns+Kunzendorf.*	*Reading* Absorbed by *Onderstepoort.*	*Newport* Absorbed by *Onderstepoort.*	*Binns* Absorbed by *Onderstepoort*	*Kunzendorf* Absorbed by *Onderstepoort.*
Onderstepoort	25,600	25,600	6,400	12,800	3,200	12,800	3,200	3,200	800	800	800	400	6,400	800	3,200	3,200	800	0	50	0	0
Reading	25,600	25,600	—	—	—	—	—	—	—	—	—	0	—			—		400	—		
Newport	800		12,800	—		—	—	—	—	—	—		50	—					6,400	—	—
Binns	800	—	—	25,600	—	0	0	0	0	0			—	0		0	0			6,400	—
Kunzendorf	25,600				3,200	—			0	0	—		—	50	—		0				400
Paratyphi C.	25,600			—	—		—	—	—		0			—	—				—		—
Sendai	12,800	—						—						0	—	—				—	—
London	1,600	—			—	400	0	0	0	0				0		—	—	—			
Anatum	1,600		—	—	—	—		—		—					0	0	0		—		—

0 — less than 1 : 50.

TABLE 12.

Antigen.	478 Serum Absorbed by: 478.	Storrs.	Copenhagen, 659.	Typhi-murium.	Abortus-equi.	Aberdeen.	*Storrs* Serum Absorbed by: 478.	Storrs.	*Copenhagen* 659 Serum Absorbed by: 478.	Copenhagen.	*Typhi-murium* Serum Absorbed by: 478.	Typhi-murium.	*Abortus-equi* Serum Absorbed by: 478.	Abortus-equi.	*Aberdeen* Serum Absorbed by: 478.	Unabsorbed Sera: 478.	Storrs.	Copenhagen.	Typhi-murium.	Abortus-equi.	Aberdeen.
478 "O"	0	0	0	0	0	800	0		0	0	0	0	0	0	0	800	1,600	1,600	800	800	0
478 type	0	0	0	0	12,800	0	0		0	0	0	0			0	12,800	6,400	12,800	25,600		50,000
478 group	0	0	0	0	3,200	0	0		0	0	0	0			0	3,200	25,600	6,400	25,600		6,400
Storrs "O"	0	0			—		0	0								800	1,600				
Storrs-type	0	0	—		—		0	0								12,800	6,400				—
Storrs-group	0	0			—		0	0								3,200	25,600				
Copenhagen "O"	0	—	0		—				0	0						800	—	1,600			—
Copenhagen-type	0	—	0			—	—	—	0	0			—			12,800		12,800		—	
Copenhagen-group	0	—	0	—	—	—			0	0				—		3,200		6,400		—	
Typhi-murium "O"	0	—	—	0		—		—			200 400	0	—	—		800			800	—	
Typhi-murium-type	0		—	100	—	—		—			100	100	—		—	12,800		—	25,600	—	
Typhi-murium-group	0		—	100		—	—				100	100	—		—	3,200	—	—	25,600	—	—
Abortus equi "O"		—		0	0	—	—	—			—	—	0	0		1,600	—	—	—	1,600	—
Abortus-equi "H"		—		—	0			—		—		—	—	—	—	—	—				
Aberdeen "O"	—	—		—	—	0		—		—		—	—	—	400	0	—		—		400
Aberdeen type	—	—	—	—	—	0	—	—		—			—		100	12,800					50,000
Aberdeen group	—	—	—	—		0	—		—	—				—	0	3,200		—			6,400

0 Less than 1 : 100. — No test carried out.

TABLE 9.—(After Henning and Greenfield.)

Antigen.	Unobserved Sera.						Absorbed Sera.								
	Afri. Aust Serum Un-Absorbed.	*Heidelberg* Serum Un-Absorbed.	*Kunzendorf* Serum Un-Absorbed.	*Newport Kottbus* Serum.	*Muenchen* Serum.	*Bovis-morbificans* Serum. Un-absorbed.	*Afri. Aust* Serum Absorbed with *Afri. Aust.*	*Afri. Aust* Serum Absorbed with *Kunzendorf.*	*Afri. Aust* Serum Absorbed with *Heidelberg.*	*Afri. Aust* Serum Absorbed with *Bovis-morbificans.*	*Kunzendorf* Serum Absorbed with *Afri. Aust.*	*Heidelberg* Serum Absorbed with *Afri. Aust.*	*Bovis-morbificans* Serum Absorbed with *Afri. Aust.*	*Bovis-morbificans* Serum Absorbed with *Bovis-morbificans.*	*Afri. Aust* Serum Absorbed by *Heidelberg* and then by *Kunzendorf.*
Afri. Aust. "O"	800	0	0	1,600	800	1,600	0	400	800	0	–		0	0	400
Afri. Aust. Type	6,400	6,400	0			6,400	0	6,400	0	0	–	–	0	0	0
Afri. Aust. Group	6,400	3,200	3,200			25,600	0	400	3,200	0	0		0	0	400
Heidelberg "O"	0	800						–	–		–	800	–		–
Heidelberg Type	6,400	6,400		–				—	0				–		
Heidelberg Group	1,600	12,800	800			–	—	–	0			6,400		–	–
Kunzendorf "O"	50	—	800	—		–	—		–		800				
Kunzendorf "H"	3,200	–	3,200	–	–		—	0			200		—	—	–
Bovis-morbificans "O"	800		0			1,600	0	—	–	0	–		0	0	
Bovis-morbificans Type	3,200	–	–	–	—	6,400	0			0			0	0	–
Bovis-morbificans Group	6,400	—	—	–		25,600	0	–		0		–	0	0	—
Newport (Kottbus) "O"	—	–	0	1,600	—	–	–		–	—	—	–	—	–	
Muenchen "O"	–	—	0	—	800	–	—	—		–	—	–	–	—	

0 – less than 1 : 50.

TABLE 20D.

Antigen.	*Anatum* S. ab. by *Anatum*.	*Anatum* S. ab. by 414.	*Anatum* S. Un-absorbed.	414 S. ab. *Anatum*.	414 S. ab. 414.	414 S. Un-absorbed.	L_2 S. ab. 414.	L_2 S. Un-absorbed.	*Kottbus* Specific S. ab. 414.	*Kottbus* Specific Unabsorbed.	414 S. ab. L_2.	414 S. ab. *Kottbus*.	414 S. a.b. *Anatum* v. *Muenster*.	414 S. ab. *Senftenberg*.	*Senftenberg* S. ab. 414.	*Senftenberg* S. Un-absorbed.
Anatum "O"	0	0	400	0	0	800	—	—			—	—	—	—		—
Anatum type	0	0	3,200	0	0	12,800				—			—	—		—
Anatum group	0	0	6,400	0	0	6,400	—		—		—	—	—	—	—	—
414—"O"	0	0	400	0	0	800	0	800	0	0	0	800	0	400	0	400
414—type	0	0	3,200	0	0	12,800	—	0	0	3,200	12,800	0	0	—	—	
414—group	0	0	6,400	0	0	6,400	—	1,600	0	800	200	800	1,600	—		—
L_2—O	—	—	—	—	—	800	0	800	—	—	0	0	—	—	—	—
L_2—type	—	—	—	—	—	0	—	320	—	—	0	—	—	—	—	—
L_2—group	—	—	—	—	—	6,400	0	6,400	—	—	0	—	—	—		—
Kottbus "O"	—	—	—	—	—	0		—	1,600	1,600	—	0	—	—	—	—
Kottbus type		—	—	—	—	3,200	—	—	0	3,200	—	0	—	—	—	—
Kottbus group	—	—	—	—	—	1,600	—	—	800	3,200	—	0	—	—	—	—
Anatum v. *Muenster* "O"	—	—	—	—	—	800	—	—	—	—	—	—	0	—		—
Anatum v. *Muenster* type	—	—	—	—	—	6,400	—	—	—	—	—	—	0	—		—
Anatum v. *Muenster* group	—	—	—	—	—	1,600	—	—	—	—	—	—	0	—	—	—
Senftenberg "O"	—	—	—	—	—	200	—	—	—	—	—	—	—	—	400	800

0 = < 1 : 100, S. = Serum, ab. = absorbed by.

TABLE 25 (continued).

Type of Organism	No. of Cultures Tested	Bitter.	Stern.	d. Tartrate (Jordan & Harmon).	No. of Cultures Tested.	Glucose.	Lactose.	Dulcite.	Saccharose.	Mannite.	Maltose.	Arabinose.	Rhamnose.	Inulin.	Inosite.	Salicin.	Galactose.	Sorbite.	Xylose.	H_2S Production.	Indol.	Litmus Milk.	Motility.	Species of Animal or Source from which Culture was obtained.
Typhi murium (Weybridge)	1	+			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Typhi-murium (Bruns)	1				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paratyphi B (Schotmuller)	1	-			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paratyphi B D.C.	1				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paratyphi B (Lowestoft)	1	- -	-		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paratyphi B (Grey)	1				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paratyphi B var *Odense* 8085	1	- -			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cholerae-suis 108 *(Kunzendorf)*	1				1																	Alk		Pig (Septicaemia)
Cholerae-suis 365 *(Kunzendorf)*	1				1																	Alk		Pig (swine fever).
Cholerae suis 381	1				1																	Alk		" "
Cholerae-suis 382	1				1																	Alk		" "
Cholerae suis 383	1				1																	Alk		" "
Cholerae suis 384	1				1																	Alk		" "
Cholerae-suis S.F. 8	1				1																	Alk		Nat. Coll. of Type Cultures
Cholerae-suis (Kunzendorf)	1				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cholerae suis (America)	1				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bovis-morbificans 391	1				1																	Alk		Pork
Bovis-morbificans (Basenau)	1				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bovis morbificans (Shahlen-Scott)	1	—			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amersfoort 338	1				1	+																Alk		Chickens.
Amersfoort 350	1				1																	Alk		Fowls.
Onderstepoort 282	1				1																	Alk		Sheep.
Aertrycke 411	1				1																	Alk		Fowl
Aertrycke 3701	1			0	1																	Alk		0
Aertrycke 3702	1		+ +	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Abortus-equi 219	1				1	⊥		⊥*		⊥	⊥	⊥	⊥				⊥	⊥	⊥			Alk	- -	Horse.
Abortus equi W.II 2	1				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gallinarum.	10	0	0		149	⊥		⊥		⊥	⊥	129⊥ 20	132⊥ 17⊥d				⊥	⊥d	⊥			Alk		Fowls 137, Turkeys 2, Chickens 10.
Gallinarum 29	1		-		1	⊥		⊥		⊥	⊥	⊥	⊥				⊥	⊥	⊥			Alk	-	Fowl
Gallinarum 360	1				1	⊥		⊥		⊥	⊥	⊥	⊥				⊥	⊥	⊥			Alk		Fowl
Gallinarum 415	1				1	⊥		⊥		⊥	⊥	⊥	⊥				⊥	⊥	⊥			Alk	-	Fowl
Gallinarum 249	1				1	⊥		⊥		⊥	⊥	⊥	⊥				⊥	⊥	⊥			Alk	- -	5 day old chickens.
Gallinarum 321	1				1	⊥		⊥		⊥	⊥		⊥				⊥	⊥	⊥			Alk		Hen.
Gallinarum 340	1		-		1	⊥		⊥		⊥	⊥	⊥	⊥				⊥	⊥	⊥			Alk		Fowl.
Pullorum	15				15	⊥				⊥		37⊥ 8	35⊥ 10⊥d				⊥	⊥d	⊥		—	Neut		Chickens and ovaries of hens.
Pullorum 207	1				1	⊥				⊥		⊥	⊥				⊥	⊥	⊥			Neut.		Chicken.
Pullorum 317	1		—		1	⊥				⊥			⊥d				⊥	⊥	⊥d			Neut	—	Chickens and ovary of hen.
Pullorum 322	1			-	1	⊥				⊥			⊥d				⊥	⊥	⊥d		- -	Neut	—	Chicken (7 day).
Pullorum 337	1	- -	- -		1	⊥			—	⊥		⊥	⊥d				⊥	⊥	⊥d			Neut	-	Chick.
Pullorum 436	1				1	⊥				⊥		⊥					⊥	⊥	⊥d		- -	Neut		Chicks

Stern: +, ++ and +++ indicate different degrees of discoloration, +++ being the most deeply dark blue colour.
d-tartrate: +, ++ and +++ indicate that ¼, ½ and ¾ respectively of the medium has been changed yellow.
Positive; ⊥ acid, but no gas is formed; ⊥d delayed fermentation.
⊥* acid, but no gas formed 4 years ago, negative now.

TABLE 25.

Fermentation reactions of the different types of Salmonella *studied.*

Type of Organism.	No. of Cultures Tested.	Bitter	Stern.	d. Tartrate (Jordan & Harmon)	No. of Cultures Tested.	Glucose.	Lactose.	Dulcite.	Saccharose.	Mannite.	Maltose.	Arabinose	Rhamnose.	Inulin	Inosite.	Salicin	Galactose	Sorbite.	Laevulose.	H. S. Production	Indol.	Litmus Milk	Motility	Species of Animal or Source from which Culture was obtained
Enteritidis var. *dublin*	50	50	50	36 / 14	97							21 / 76	79 / 18									Alk	91 / 3	Calves 96, Human 1
Dublin 154	1				1																	Alk		Calf
Dublin 170	1				1			+														Alk		Calf
Dublin 175	1		—		1																	Alk		Calf
Dublin 283	1				1																	Alk		Calf
Dublin 295	1				1																	Alk		Calf
Dublin 303	1				1																	Alk		Calf
Dublin 329	1				1																	Alk		Calf.
Dublin 312	1				1																	Alk		Calf
Dublin 441	1				1																	Alk		Calf
Dublin 415	1				1		—															Alk		Calf
Dublin Pesch 258	1				1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dublin Knox (2206 A)	1				1																	Alk		0
Dublin Cambridge I	1				1																	Alk		Calf
Dublin Topley	1				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paracoli strain 255	1				-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enteritidis 216	1				1																	Alk		Calf.
Enteritidis 290	1				1																	Alk		Calf.
Enteritidis 118	1				1																	Alk		Calf
Enteritidis M. 7	1				1																	Alk		0
Enteritidis D. 5	1				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enteritidis Weybridge	1				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enteritidis Jena	1				0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0
Enteritidis var. *Rostock* 3747	1				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enteritidis var. *Moscow*	1				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enteritidis var. *Blegdam*	1				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enteritidis var. *Essen*	3				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0		0
Typhi	2				2	⊥				⊥	⊥						⊥	⊥	⊥			Neut		0
Typhi 208	1				1	⊥		⊥		⊥	⊥						⊥	⊥	⊥			Neut		Chicken
Typhi Tp2	1				1	⊥				⊥	⊥						⊥	⊥	⊥			Neut		0
Typhi murium	13	12	13	5 / 8	10								9		8							Alk		0
Typhi-murium 190	1				1																	Alk		Calf
Typhi murium 192	1				1									0	0							Alk		Pig
Typhi-murium 176	1				1																	Alk		Canary.
Typhi murium 177	1				1																	Alk		Grain for canary.
Typhi-murium 357	1				1																	Alk		Chickens
Typhi murium 502	1				1																	Alk		Calf
Typhi murium 234	1				1																	Alk		Sheep.
Typhi murium var. *Storrs* 478, *Copenhagen*	1				1																	Alk		Foal (arthritis)
Typhi murium V. *Storrs* 10500	1				1																	Alk		0
Typhi-murium V. *Copenhagen* 659	1				1																	Alk		0
Typhi-murium V. *Copenhagen* 1147	1				1																	Alk		0
Typhi murium (*Glasgow*)	1				1													+				Alk		0
Typhi murium (*Breslau*)	1				1																	Alk		0
Typhi murium (*Mutton*) 74	1				1																	Alk		0

TABLE II.

Summary of Data on Weight Loss and Food Intake of Groups of Rats during First and Second Trials on Various Defective Diets and Starvation.

Number of Experiment.	Number of Animals.	Sex.	Dietary Deficiencies.	Trial.	Weight Grams.	Average change in body weight *: For first 12 days. Grams.	Average change in body weight *: For first 12 days. Per cent.	Average change in body weight *: For first 24 days. Grams.	Average change in body weight *: For first 24 days. Per cent	Grand average food consumption: For first 12 days. Per Rat per day Grams.	Grand average food consumption: For first 12 days. Per 100 gm. Rat per day Grams.	Grand average food consumption: For first 24 days. Per Rat per day Grams.	Grand average food consumption: For first 24 days. Per 100 gm. Rat per day Grams.
I.	6	Males	Vitamins and minerals	First	237·7	14·4	— 6·1	- 31·0	13·0	8·6	3·7	7·4	3·3
			Vitamins and minerals	Second	251·8	+ 3·5	1·4	– 5·3	2·1	11·8	4·6	10·3	4·1
	6	Females	Vitamins and minerals	First	178·4	6·4	3·6	- 19·0	10·6	7·3	4·2	6·4	3·8
			Vitamins and minerals	Second	211·0	9·4	4·4	39·4	18·7	8·9	4·2	6·4	3·2
II.	12	Males	Vitamins and minerals	First	282·4	- 15·0	5·3	29·2	10·3	9·4	3·4	8·1	3·0
			Vitamins and minerals	Second	295·5	4·0	1·3	- 20·7	7·0	10·6	3·6	9·0	3·1
	12	Females	Vitamins and minerals	First	205·7	7·7	3·7	23·4	11·4	7·3	3·6	6·2	3·1
			Vitamins and minerals	Second	207·7	12·2	5·9	26·2	12·6	6·3	3·2	5·5	2·8
III.	6	Males	Vitamins, minerals and bulk	First	256·7	2·5	1·0	10·9	4·2	10·7	4·2	9·9	3·9
			Vitamins, minerals and bulk	Second	256·8	+ 7·9	3·1	1·1	0·4	11·6	4·4	10·6	4·0
	6	Females	Vitamins, minerals and bulk	First	172·8	- 2·5	1·5	5·3	3·1	7·6	4·5	7·5	4·4
			Vitamins, minerals and bulk	Second	179·5	0·5	0·2	9·3	5·2	8·1	4·5	7·0	4·0
IV.	6	Males	Vitamins, minerals and bulk	First	273·0	8·5	3·1	10·3	3·8	9·5	3·6	9·2	3·5
			*Vitamins, minerals and bulk	Second	282·8	+ 4·4	+ 1·6	0·5	0·2	10·6	3·7	9·6	3·4
	6	Females	Vitamins, minerals and bulk	First	211·5	- 5·5	2·6	12·4	5·9	8·8	4·2	8·0	3·9
			Vitamins, minerals and bulk	Second	212·5	1·5	0·7	9·0	4·2	8·6	4·1	7·9	3·7
V.	6	Males	Proteins	First	232·8	38·1	-16·4	52·8	- 22·7	9·3	4·3	9·1	4·6
			Proteins	Second	236·0	33·1	-14·0	50·6	- 21·4	8·7	3·9	8·6	4·2
	6	Females	Proteins	First	198·9	- 28·2	14·2	-41·9	21·1	7·9	4·2	7·6	4·4
			Proteins	Second	199·3	—25·9	- 13·0	— 39·2	19·7	7·8	4·2	7·5	4·3
						For first	8 days.	For first	16 days.				
VI.	6	Males	Starvation but water allowed	First fast	409·4	79·8	—19·5	—140·1	34·2	—	-	-	—
			Starvation but water allowed	Second fast	401·6	-75·5	– 18·8	—124·8	- 31·1	-	-		-
	6	Females	Starvation but water allowed	First fast	298·9	57·7	—19·2	—99·3	– 33·2	—	—	—	
			Starvation but water allowed	Second fast	290·3	– 57·1	—19·7	-92·7	- 31·9	—	—	-	

* The signs — and + signify respectively loss and gain in body weight.

TABLE 9

Experiments 9 (a) and 9 (b). To test the duration of immunity in sheep that recovered from the "Mara" and "Strydom" strain of heartwater.

D.O.B. Number of Sheep	Injected with.	Expt. S.	Date of Injection.	Incub. Period in Days.	Duration of Disease in Days.	Result.	Interval in Days between Injection and 1st Immunity Test.	First Immunity Test. Injected with	First Immunity Test. Date of Injection.	First Immunity Test. Result.	Interval between Injection and 2nd Immunity Test. Number of Days.	Interval between Injection and 2nd Immunity Test. Approximate Number of Months	Second Immunity Test. Injected with	Second Immunity Test. Date of Injection.	Second Immunity Test. Incub. Period	Second Immunity Test. Duration of Disease	Result.
51473	Strydom HW. strain	6547	20/10/38	8	10	Recovered	32	Mara HW. strain	21/11/38	Reacted to virus "A"	284	9	Strydom HW. strain	31/7/39			No reaction.
51631	Strydom HW. strain	6547	20/10/38	10	9	Recovered	32	Mara HW. strain	21/11/38	Reacted to virus "A"	284	9	Strydom HW. strain	31/7/39			No reaction.
52794	Strydom HW. strain	6547	8/10/38	10	8	Recovered	44	Mara HW. strain	21/11/38	No reaction	296	10	Strydom HW. strain	31/7/39	16	3	Febrile reaction 106 F. No clinical symptoms.
46098	Mara HW. strain	6031	3/3/37	13	15	Recovered	42	Mara HW. strain	15/4/37	No reaction.	837	28	Mara HW. strain	31/7/39			No reaction
45974	Mara HW. strain	6020	16/10/36	13	15	Recovered	36	Mara HW. strain	21/11/36	No reaction	1,018	34	Mara HW. strain	31/7/39	11	3	Febrile reaction 105·6. No clinical symptoms.
43659	Mara HW. strain	6020	18/9/36	6	9	Recovered	64	Mara HW. strain	21/11/36	No reaction.	1,046	35	Mara HW. strain	31/7/39			No reaction.
43749	Mara HW. strain	5688	23/6/35	8	13	Recovered	23	Northam HW. strain	15/7/35	No reaction.	1,475	49	Mara HW. strain	31/7/39			No reaction.
41021	Mara HW. strain	5688	23/4/35	8	12	Recovered	68	Northam HW. strain	29/6/35	No reaction	1,580	52	Mara HW. strain	31/7/39			No reaction.
41016	Mara HW. strain	5507	27/10/34	13	14	Recovered	769	Mara HW. strain	4/12/36	No reaction	1,738	58	Mara HW. strain	31/7/39			No reaction.

TABLE 8 (*a*)

The infection with heartwater of the 13 control sheep which were kept together with the heartwater recovered sheep in a camp

D.O.B. Number of Sheep.	Injected with Heartwater Strain.	Expt. S.	Date of of Injection.	Incubation Period in Days.	Duration of Disease	Remarks
40955	" Mara ".............	5263	31/7/39	11	6	Died. *Rickettsia ruminantium* found in the intima smears
42993	" Mara ".............	5263	31/7/39	8	8	Died. *Rickettsia ruminantium* found in the intima smears
43126	" Mara ".............	5263	31/7/39	8	7	Died. *Rickettsia ruminantium* found in the intima smears
45956	" Mara ".............	6031	31/7/39	8	7	Died *Rickettsia ruminantium* found in the intima smears
40306	" Mara ".............	6730	31/7/39	9	10	Died. *Rickettsia ruminantium* found in the intima smears
47071	" Mara ".............	6730	31/7/39	10	8	Died. *Rickettsia ruminantium* found in the intima smears
48769	" Mara ".............	6730	31/7/39	11	7	Died. *Rickettsia ruminantium* found in the intima smears
45928	" Mara ".............	6730	31/7/39	10	5	Died. *Rickettsia ruminantium* found in the intima smears
49050	" Mara ".............	6730	31/7/39	11	4	Died. *Rickettsia ruminantium* found in the intima smears
55452	" Mara ".............	6711	31/7/39	7	15	Recovered.
54526	" Mara ".............	6711	31/7/39	8	15	Recovered.
54913	" Strydom "...........	6710	31/7/39	8	12	Recovered.
55536	" Strydom "...........	6710	31/7/39	9	7	Died. *Rickettsia ruminantium* found in the intima smears

TABLE 8.

Experiment 8.—To test the duration of immunity in sheep that recovered from the "Mara" strain of heartwater.

D.O.B. Number of Sheep.	Injected with	Expt. S.	Date of Injection.	Incub. Period in Days.	Duration of Disease in Days.	Result	Interval between Infection and Immunity Test. Number of Days.	Interval between Infection and Immunity Test. Approximate Number of Months.	Immunity Test. Injected with	Immunity Test. Date	Immunity Test. Incub. Period in Days	Immunity Test. Duration of Disease in Days	Remarks.
51424	Mara HW. strain	6103	19/ 4/39	12	17	Recovered	104	3	Mara HW. strain	31/ 7/39	—	-	No reaction
50059	Mara HW. strain	6599	13/ 2/39	10	12	Recovered	168	5	Mara HW. strain	31/ 7/39			No reaction
51449	Mara HW. strain	6571	13/ 2/39	17	14	Recovered	168	5	Mara HW. strain	31/ 7/39			No reaction
52748	Mara HW. strain	6607	21/ 1/39	10	15	Recovered	191	6	Mara HW. strain	31/ 7/39			No reaction
52705	Mara HW. strain	6607	21/ 1/39	10	14	Recovered	101	6	Mara HW. strain	31/ 7/39			No reaction
52632	Mara HW. strain	6571	9/ 1/39	11	8	Recovered	203	7	Mara HW. strain	31/ 7/39	11	11	Febrile reaction 108 F. No clinical symptoms.—Recovered.
51386	Mara HW. strain	6559	21/11/38	7	15	Recovered	252	8	Mara HW. strain	31/ 7/39			No reaction.
52126	Mara HW. strain	6531	10/11/38	7	12	Recovered	263	9	Mara HW. strain	31/ 7/39	—		No reaction.
51683	Mara HW. strain	6531	31/10/38	7	14	Recovered	274	9	Mara HW. strain	31/ 7/39	–	-	No reaction.
41498	Mara HW. strain	5527	28/ 7/38	11	10	Recovered	369	12	Mara HW. strain	31/ 7/39	10	10	Febrile reaction 107 F. No clinical symptoms.—Recovered.
49384	Mara HW. strain	5527	1/ 6/38	8	17	Recovered	395	13	Mara HW. strain	31/ 7/39		-	No reaction.
49979	Mara HW. strain	5527	27/ 6/38	8	7	Recovered	401	13	Mara HW. strain	31/ 7/39			No reaction
49929	Mara HW. strain	5527	14/ 6/38	10	12	Recovered	412	13	Mara HW. strain	31/ 7/39		—	No reaction.
50384	Mara HW. strain	5527	21/ 5/38	6	20	Recovered	437	14	Mara HW. strain	31/ 7/39	-		No reaction.
49681	Mara HW. strain	5527	11/ 5/38	6	22	Recovered	447	15	Mara HW. strain	31/ 7/39			No reaction.
49386	Mara HW. strain	5527	19/ 4/38	9	11	Recovered	468	15	Mara HW. strain	31/ 7/39	—		No reaction
50319	Mara HW. strain	6400	19/ 4/38	8	10	Recovered	468	15	Mara HW. strain	31/ 7/39		-	No reaction.
50306	Mara HW. strain	5527	26/ 3/38	10	17	Recovered	492	16	Mara HW. strain	31/ 7/39	13	8	Febrile reaction 107 F. No clinical symptoms. Recovered.
50360	Mara HW. strain	5527	9/ 2/38	9	20	Recovered	538	18	Mara HW. strain	31/ 7/39	—		No reaction.
49876	Mara HW. strain	5527	12/ 1/38	6	21	Recovered	565	19	Mara HW. strain	31/ 7/39		—	No reaction
46124	Mara HW. strain	6031	15/ 4/37	9	14	Recovered	837	28	Mara HW. strain	31/ 7/39	-		No reaction
42531	Mara HW. strain	6031	1/ 3/37	10	13	Recovered	882	29	Mara HW. strain	31/ 7/39			No reaction
43482	Mara HW. strain	6031	1/ 3/37	12	11	Recovered	882	29	Mara HW. strain	31/ 7/39			No reaction
45747	Mara HW. strain	5527	19/ 2/37	8	19	Recovered	893	29	Mara HW. strain	31/ 7/39		-	No reaction.
46369	Mara HW. strain	5527	8/ 2/37	10	21	Recovered	903	30	Mara HW. strain	31/ 7/39	17	8	Febrile reaction 107.4 F. No clinical symptoms.—Recovered.
43391	Mara HW. strain	6096	8/ 2/37	8	15	Recovered	903	30	Mara HW. strain	31/ 7/39	11	3	Febrile reaction 105°F. No clinical symptoms. Recoverved.
46715	Mara HW. strain	6095	8/ 2/37	10	12	Recovered	903	30	Mara HW. strain	31/ 7/39	—	-	No reaction.
47029	Mara HW. strain	6095	8/ 2/37	9	8	Recovered	903	30	Mara HW. strain	31/ 7/39			No reaction.
43755	Mara HW. strain	6104	7/ 1/37	11	14	Recovered	935	31	Mara HW. strain	31/ 7/39		-	No reaction
47078	Mara HW. strain	5527	30/ 9/36	9	24	Recovered	1,035	34	Mara HW. strain	31/ 7/39			No reaction
46230	Mara HW. strain	6011	10/ 9/36	7	15	Recovered	1,054	35	Mara HW. strain	31/ 7/39			No reaction
44586	Mara HW. strain	5623	9/ 3/36	7	12	Recovered	1,239	41	Mara HW. strain	31/ 7/39	—	-	No reaction.

TABLE 7.

Experiment 7 (a) and 7 (b) (S.4377, S.5722 and S.6045).—To test the immunity in sheep that recovered from "S.4377" strain against "Mara" heartwater strain.

D.O.B. Number of Sheep.	Date of Injection.	Heartwater Strain.	Result.	First Immunity Test.					Interval in Days between Artificial Infection and 2nd Immunity Test.	Interval in Days between 1st and 2nd Immunity Test.	Second Immunity Test.			
				Interval in Days between Artificial Infection and Immunity Test.	Heartwater Strain.	Date of Injection.	Dose of Blood i.v.	Result.			Heartwater Strain.	Date of Injection.	Dose of Blood i.v.	Result.
40936	28/8/34	"S. 4377"	Recovered from heartwater	320	"Mara"	13/7/35	10 c.c.	No reaction	773	444	"Mara"	30/9/36	10 c.c.	No reaction.
41107	19/10/34	"S. 4377"	Recovered from heartwater	236	"Mara"	13/7/35	10 c.c.	No reaction	680	444	"Mara"	30/9/36	10 c.c.	No reaction.
41552	14/12/34	"S. 4377"	Recovered from heartwater	211	"Mara"	13/7/35	10 c.c.	No reaction	655	444	"Mara"	30/9/36	10 c.c.	No reaction.
41537	3/12/34	"S. 4377"	Recovered from heartwater	222	"Mara"	13/7/35	10 c.c.	No reaction	666	444	"Mara"	30/9/36	10 c.c.	No reaction
39205	10/9/34	"S. 4377"	Recovered from heartwater	306	"Mara"	13/7/35	10 c.c.	No reaction	750	444	"Mara"	30/9/36	10 c.c.	On the 20th day after the injection sheep showed a febrile reaction which lasted 5 days. On further tests it was found that this reaction was produced by "virus A.".
39853	28/9/34	"S. 4377"	Recovered from heartwater	288	"Mara"	13/7/35	10 c.c.	No reaction	732	444	"Mara"	30/9/36	10 c.c.	On the 8th day after injection sheep showed a febrile reaction which lasted for 12 days. The highest temperature recorded was 105·2° F. On the 10th day of the reaction blood was injected into 2 susceptible sheep 46439 and 45823. Both these animals developed heartwater and died. The febrile reaction in the sheep therefore was due to heartwater.

Table No. 3.

Pica.

Group.	No. of Animal.	14	28	42	56	70	84	98	112	126	140	154	168	182	196	210	224	238	252	266	280	294	308	322	336	350	364	378	392	406	420	434	448	462	476	Total. ×	Total. ○	Grand Total.
				Age of Calves in Days.																																		
				Bone supplement from 30 days onwards.																																		
Bonemeal	6727																																					
Bonemeal	6730																																					
Bonemeal	6736																																					
Bonemeal	6740																																					
Bonemeal	6761																																					
Bonemeal	6773																																			3		3
Bonemeal	6777																																				1	1
Bonemeal	6789															○																				1		1
Bonemeal	6790																																			1	3	4
Totals																1	1									1			1	1	2	2				5	4	9
																				Received bonemeal from 271 days onwards.																		
Control	6714																																			1	1	2
Control	6728																																			1	10	11
Control	6742																																			1		1
Control	6743																																			7	9	16
Control	6748																																			19	5	24
Control	6749																																			14	5	19
Control	6768																																			12	4	16
Totals						1	2	4	3	3	4	4	5	5	5	5	5	5	5	5	4	5	6	2	1	1		1	2	2		1	1	1	1	54	34	88

Table No. 6.

Section No. I.

Body Weights at Birth and thereafter Monthly. Milk in Amount per Head per day for the Month. All figures in lb.

Group.	No. of Calf.	No. of Dam.	Degree of Breeding.	Sex.	Date of Birth.	Criteria.	Birth.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Bonemeal Control	6714	3117	7/8	M.	18/11/36	Body weight	66	106	150	202	238	275	292	310	313	320		337	380	377	463	488	555
						Milk per day		7·68	7 73	8·34	7·10	5·30	3·49	2 21									
Bonemeal Control	6742	5518	7/8	M.	7/12/36	Body weight	81	118	163	221	242	278	295	286	298	299	312	349	352	395	461	538	597
						Milk per day		8·85	11 10	9·68	6·28	4 69	2 87	2 33									
Bonemeal Control	6743	4941	7/8	M.	7/12/36	Body weight	77	115	166	216	260	299	314	313	328	331	345	395	380	424	489	572	600
						Milk per day		8·36	10·04	9·04	6·81	5 71	3·20	2·57									
Bonemeal Control	6748	3037	7/8	M.	8/12/36	Body weight	67		143	196	237	266	270	269	273	273	269	299	304	355	420	475	540
						Milk per day		7·66	7·97	6 69	4·94	3 72	1 88	1 35									
I. Body Weights— (a) Totals							291	339	622	835	977	1,118	1,171	1,178	1,212	1,223	926	1,390	1,416	1,551	1,833	2,073	2 292
(b) Averages							73	113	156	209	244	280	293	295	303	306	309	348	354	388	458	518	573
II. Milk— (a) Total milk per group per day							--	32·55	36·84	33·75	25·13	19·42	11 44	8 47									
(b) Average milk per calf per day								8·14	9·21	8 44	6 28	4 86	2 86	2 12									

Section No. II.

Group.	No. of Calf.	No. of Dam.	Degree of Breeding.	Sex.	Date of Birth.	Criteria.	Birth.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Straight Control	6509	3242	7/8	M.	11/11/35	Body weight	57	86	128	168	201	226	261	267	270	261	278	271	292	300	360	380	439
						Milk per day		3·89	5 03	5 31	4 33	3 16	2 09	1 32									
Straight Control	6611	3139	7/8	M.	22/12/35	Body weight	64	95	115	143	177	202	210	213	213	212	215	235	246	305	285	320	315
						Milk per day		6 40	1 19	3 80	2 81	1 85	0 90	0 53									
Straight Control	6759	4977	7/8	M.	13/12/36	Body weight	77	100	138	175	222	232	259	260	265	285	286	303	303	353	364	140	487
						Milk per day		7·65	8·31	6 28	1 50	2 73	1 30	1 53									
I. Body Weights— (a) Totals							198	281	381	481	600	670	730	740	748	758	779	809	841	958	1,000	1,158	1,241
(b) Averages							66	94	127	161	200	223	243	247	249	253	260	270	280	319	336	386	414
II. Milk— (a) Total milk per group per day								17 94	17·83	15·39	11·64	7 73	4·29	3 38									
(b) Average milk per calf per day								5·98	5 94	5·13	3 88	2 58	1 43	1 13									

Section No. III.

Criteria	Birth.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Body Weights— (a) Actual difference between groups	+ 7	+ 19	+ 29	+ 48	+ 44	+ 57	+ 50	+ 48	+ 54	+ 53	+ 49	+ 78	+ 74	+ 69	+ 122	+ 132	+ 159
(b) Percentage difference between groups	10·61	**20·21(5)**	**22·83(5)**	**29 81(1)**	**22 00(5)**	**25 56(1)**	**20 58(5)**	**19·43(5)**	**21 69(5)**	20 95	18·85	**28 89(5)**	**30 00(5)**	**21 63(5)**	**36·31(1)**	**34·20(5)**	**38·41(5)**
Milk— (a) Actual difference between groups		2·16	3·27	3 31	2 40	2·28	4·43	0 99									
(b) Percentage difference between groups		36 12	55·05	**64·52(5)**	**61 86(5)**	**88 37(5)**	**100 00(5)**	87 86									

Section No. IV.—Body Weights Corrected for Birth Weights.

Criteria	Birth.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Body Weights— (a) Averages for "Bonemeal-control" group	-	109·9	153·0	205·7	240·5	276·6	290·2	294 3	301 1	301·7	299·9	330·2	350 5	379·1	451·5	505·1	561·2
(b) Averages for "Straight-control" group	-	97·1	130·7	165·1	204 3	227 2	246 4	247 8	251·4	258 3	260·1	279 6	284·5	329·9	341·6	401·9	428·6
(c) Actual differences	—	12·8	22·4	40·6	36 2	49·4	43·8	46 5	49 7	43·4	39 8	60·6	66 0	49·2	111·9	103·2	132·6
(d) Percentage differences	—	**13·18(1)**	**17·13(5)**	**24·55(1)**	**17·72(5)**	**27 74(5)**	17·78	18 77	19 77	16·80	11·45	21·67	23·20	**14·91(5)**	**32·80(5)**	**25·68(5)**	**30·94(5)**

Section No. V.—Body Weights Corrected for Milk Supply.

Criteria	Birth.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Body Weights— (a) Averages for "Bonemeal-control" group	-	108·0	148·5	194·4	232·0	264·3	277·4	277·6		-		—	—	—	—	—	—
(b) Averages for "Straight-control" group	—	99·0	137·0	180·5	216·0	243·8	263·9	269·6			—		—	—	—	—	—
(c) Actual differences	-	9·0	11·5	13·9	16·0	20·5	13·5	8·0			—		—	—	—	—	—
(d) Percentage differences	—	9·09	8·39	7·70	7·41	8·43	5·12	2·97				—	—	—	—	—	—

N.B.—(5) Indicates significances P. = 0·05; (1) Indicates significances P. = 0·01.

TABLE 34.

Correlated Development.

Age in Weeks	External Appearance.	Head and Face.	Eye.	External Ear.	Genitalia.	Limbs	Hair and Wool.
3rd....	Whitish, translucent comma-shaped. Brain regions developing. Heart pinhead-sized focus. Aorta and umbilical vessels visible	—	—	—	—		
4th ...	Heart 0·5 cm. diameter. Aorta and carotids visible. Cranial region large. Tail 2 mm.		Thin black ring 1 mm. diameter	–		Both pairs of buds seen Thoracic 3 mm. long	
5th......	Vertebral column foreshadowed. Surface pink. Heart still visible. Ventral abdominal bulge		Diameter of ring 1·5 mm. Pigment more distinct	Triangular flap, 1 mm. in length		Cent. constriction. Division into digits. Distal part rotated	
6th.. ...	Definitive mammalian features evident. Heart invisible. Cart. models of vertebrae and scapulae seen	Mouth and nostrils distinguishable	Diameter 3 mm. Lens opaque. Lids become visible	Length of flap increased to 1·5 mm		Digits distinct. Signs of access. digits Division of limb plainly visible	Pinhead sized white foci on eyelids and lips
7th	Head large, crown prominent. Neck still sharply bent	Mouth and nostrils plainly visible	Diameter 5 mm. Lids over eye. Signs of infra. orb. pouch	About 2 mm. in length		Coronary bands seen as transverse ridgelike swellings	Above foci recognisable as follicles of tactile hairs. Similar foci above eyes and spreading on to crown region.
8th... ...	Neck long, thin and straight. Subcut. Vessels no longer visible		Eye protruding from head. Pouch distinct	Flap 3 mm. Central longitudinal ridge present	Scrotal and labial swellings visible, also penis and sheath	Fetlock region taking shape Coronet more prominent	Ordinary hair follicles over fore-arm and in axilla Also seen on anterior aspect of carpus Little later also on posterior surface of hock
9th.. ..	Proportions of body become improved. Head still large	"Dished" profile less exaggerated	Ring wide and more deeply situated		Slight advance on above		Tactile follicles large Ordinary ones over whole body, sparser towards caudal extremity.
10th......	–	Face increased in relative size	Diameter 1·5 cm. Width 5 mm Lids plain	Appear. of ext. meatus. 3 ridges present	Labial swellings migrated analwards	Horn deposited just below coronary band	Tactile hairs not erupted, but elevate surface
11th... .	—	Face has its definitive appearance. Horn buds visible	Ring no longer clearly visible		Penis and prepuce fairly well developed		Tactile hairs just emerging at a slant Ordinary follicles closely packed over fore-quarters.
12th .. .		-	Ring only faintly visible. Lid thick	–	Labial swellings fairly near anal region		
13th..	Head still relatively large. Neck thin		Ring invisible	Flap length 2·5 cm	Folds in definitive position	Appearance of interdig. pouch	Tactile hairs up to 6 mm. long. Ordinary hairs erupting on crown. Whorls round horn-bud.
14th.	Thickness of neck increasing. Skin on neck wrinkled	External nares open. Teeth Anlagen visible	—				Hairs on forehead visible to naked eye Those on coronet and forearm seen with lens.
15th.	Similar wrinkles on brisket, and extending towards forearm	--	Lids well developed, still fused		3 mm. hairs around opening of prepuce	–	Forehead covered with well-matted hair. That on hindquarters just visible
16th...	Wrinkles visible on forearm	Philtrum of upper lip distinct	Infra-orbital pouch well developed	-	Preputial hairs 3 cm. Scrotum wrinkled		Tactile hairs up to 1 cm in length On lower part of face hair just visible with the naked eye.
17th..	Wrinkled appearance extending over forequarters	—	—	Flap 4·5 cm. 5 longitudinal ridges	Teats 6 mm. in length	Interdigit pouches well formed	Entire body well covered with white, glistening hair. Shorter posteriorly.
18th...	–	Edges of nostrils bare and smooth	Eyelids no longer fused	—	—	Horn deposition as far as heels	In neck region a dull white curly coat appears below the hairy covering.
19th.	Most of skin appears loose and wrinkled; less so posteriorly		—	—	Scrotum collapsed	Horn present on sole and access. digits	Curly coat more distinct on neck and also detected on anterior half of trunk.
20th...	Skin over entire body extremely loose and wrinkled	Inner edges of lips serrated	—	-	Prepuce well covered with hair	Deposition of horn farther advanced	Curly "under coat" on entire trunk. Straight hairs sparser and fairly loose.
21st...... .	–	1st pair Incisors about to erupt	—	Flap 6 cm. in length........	Vagina well developed	Hoof and access. digits covered with horn	Hair present only on lower face and distal halves of limbs, remainder of surface has woolly coat.